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Volume LXI Number 1

THE BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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AUGUST, 1931

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ANNOUNCEMENT

AN INDEX of the Biological Bulletin, Volumes 1 to 60, will be published in October 1931. This Index will contain an alphabetical list of authors, showing the titles of their papers, and a classified index of subjects. As the volumes of the Biological Bulletin have contained no indexes prior to 1930, this Index should add greatly to the convenience of utilizing the volumes published during this period.

The Index will also cover the material included in the Zoological Bulletin, Volumes 1 and 2.

The edition of the Index is limited. The filling of orders received after October first cannot be guaranteed.

The price of the Index is $4.00 postpaid. Orders should be sent to the Marine Biological Laboratory, Woods Hole, Mass.

Vol. LXI, No. 1 August, 1931

THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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FORTY-THIRD YEAR

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years. There shall be thirty-two Trustees thus chosen divided into four classes, each to serve four years, and in addition there shall be two

groups of Trustees as follows: (*a*) Trustees *ex officio*, who shall be the President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer and the Clerk; (*b*) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next annual meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee Emeritus for life. The Trustees *ex officio* and Emeritus shall each have the same right to vote as the regular Trustees.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen: Herewith is submitted my report as Treasurer of the Marine Biological Laboratory for the year 1930.

The accounts have been audited by Seamans, Stetson and Tuttle, certified public accountants. A copy of their report is on file at the Laboratory and is open to inspection by members of the Corporation.

At the end of the year 1930, the book value of the General Endowment Fund in the hands of the Central Hanover Bank and Trust Company (of New York) as Trustee was $908,915 in securities and $34.50 in cash. The actual market value of the securities in this fund on the 9th day of May taking the mortgages at face value, was $931,981.25, showing a very satisfactory appreciation of the value over cost.

The book value of the Library Fund was $199,922.50 in securities and $77.50 in cash. The actual market value of the securities on May 9th was $203,031.25.

At the end of the year the Lucretia Crocker Fund consisted of securities of the book value of $4,707.59 and $374.32 in cash.

The Bio Club Scholarship Fund consisted of a mortgage participation of $2,000 and cash of $31.28, the Reynold A. Spaeth Memorial Lecture Fund of $3,000 in mortgage securities and cash of $75.46.

The Reserve Fund, consisting of the proceeds of the sale of part of the Bar Neck property to the Woods Hole Oceanographic Institution, consisted at the end of the year of bonds of the book value of $20,868.75 and cash of $3,090.55, of which cash $3,000 was later paid out under the contract, leaving net proceeds of the transaction of $20,959.30 which is being held with its income to meet maturing mortgage obligations or for such other purposes as the Trustees may decide.

The Retirement Fund at the end of the year consisted of $15,800 invested in mortgage participations, less an overdraft of $9.73, leaving $15,790.27.

The land, buildings, equipment and library, excluding the Devil's Lane and Gansett property, represented an investment of $1,617,086.71, less depreciation of $246,625.64, or a net amount of $1,370,461.07.

Current expenses including depreciation exceeded income for the year by $3,767.25.

Over $29,000 was expended from current funds on buildings, equipment and library.

At the end of the year the Laboratory owed $1,640.99 on accounts payable and $27,000 on bonds secured by mortgage.

Following is the Balance Sheet as of December 31, 1930, and the condensed statement of income and outgo for the year, also the surplus account.

EXHIBIT A

MARINE BIOLOGICAL LABORATORY BALANCE SHEET, DECEMBER 31, 1930

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank & Trust Company (of New York) Trustee—Schedules I-a and I-b		$1,108,949.50	
Securities and Cash—Minor Funds—Schedule II		10,188.65	$1,119,138.15
Plant Assets:			
Land—Schedule IV	$ 97,103.05		
Buildings—Schedule IV	1,207,354.03		
Equipment—Schedule IV	155,222.28		
Library—Schedule IV	157,407.35	$1,617,086.71	
Less Reserve for Depreciation		246,625.64	
		$1,370,461.07	
Securities and Cash in Reserve Fund		23,675.43	
Cash in Dormitory Building Fund		818.96	$1,394,955.46
Current Assets:			
Cash		$ 18,010.39	
Accounts—Receivable		18,902.69	
Inventories:			
Supply Department	$ 29,063.54		
Biological Bulletin	7,951.85	37,015.39	
Investments:			
Devil's Lane Property	$ 37,780.91		
Gansett Property	2,273.34		
Stock in General Biological Supply House, Inc.	12,700.00		
Retirement Fund Assets	15,790.27	68,544.52	
Prepaid Insurance		3,992.51	$146,465.50

Liabilities

Endowment Funds:			
General Endowment Funds—Schedule III		$1,108,949.50	
Minor Endowment Funds—Schedule III		10,188.65	$1,119,138.15
Plant Funds:			
Donations and Gifts—Schedule III		$1,025,548.61	
Other Investments in Plant from Gifts and Current Funds		364,406.85	
		$1,389,955.46	
Mortgage, Danchakoff Estate		2,000.00	
Accrued Charges on Sale of Bar Neck Land		3,000.00	$1,394,955.46

Current Liabilities and Surplus:			
Mortgage, Devil's Lane Property		$ 25,000.00	
Accounts—Payable		1,640.99	
Woods Hole Oceanographic Institution:			
Amount received for Purchase of Books for their Library	$2,500.00		
Less Expenditures	2,147.07	352.93	
Items in Suspense (Net)		70.49	
		$ 27,064.41	
Current Surplus—Exhibit C		119,401.09	$146,465.50

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1930

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 48,020.46		$ 48,020.46
Library Fund		9,270.24		9,270.24
Gifts		500.00		500.00
Instruction	8,110.03	10,230.00		2,119.97
Research	4,069.37	16,261.06		12,191.69
Evening Lectures	135.48		135.48	
Biological Bulletin and Membership Dues	7,557.17	9,421.18		1,864.01
Supply Department—Schedule V	62,030.00	62,162.82		132.82
Mess—Schedule VI	30,943.36	32,973.28		2,029.92
Dormitories—Schedule VII	31,188.42	13,764.28	17,424.14	
(Interest and Depreciation charged to above three Departments. See Schedules V, VI, and VII)	*35,424.79*			35,424.79
Dividends, General Biological Supply House, Inc.		2,540.00		2,540.00
Rent, Danchakoff Cottages	634.11	1,039.00		404.89
Rent, Microscopes		462.50		462.50
Rent, Garage, Railway, etc.		154.90		154.90
Rent, Newman Cottage	137.27	150.00		12.73
Rent, Janitor's House	35.84	422.50		386.66
Sale of Duplicate Library Sets		2,198.13		2,198.13
Interest on Bank Balances		529.87		529.87
Sundry Items		10.64		10.64
Maintenance of Plant:				
New Laboratory Expense	16,839.26		16,839.26	
Chemical and Special Apparatus	10,783.01		10,783.01	
Maintenance, Buildings and Grounds	9,892.82		9,892.82	

Library Department Expenses	8,912.66		8,912.66	
Carpenter Department Expenses	1,516.91		1,516.91	
Truck Expenses	851.48		851.48	
Sundry Expenses	772.69		772.69	
Bar Neck Property Expenses	162.54		162.54	
Workmen's Compensation Insurance	592.59		592.59	
General Expenses:				
Administration Expenses	14,509.68		14,509.68	
Endowment Fund Trustee	787.50		787.50	
Interest on Loans	120.00		120.00	
Bad Debts	317.98		317.98	
Naples Zoological Station, for Research	250.00		250.00	
Mosquito Fund Contribution	100.00		100.00	
Reserve for Depreciation	38,052.73		38,052.73	
Excess of Expenses over Income carried to Current Surplus—Exhibit C		3,767.25		3,767.25
	$213,878.11	$213,878.11	$122,021.47	$122,021.47

EXHIBIT C

MARINE BIOLOGICAL LABORATORY, CURRENT SURPLUS ACCOUNT

YEAR ENDED DECEMBER 31, 1930

Balance, January 1, 1930		$119,933.29
Add:		
Reserve for Depreciation charged to Plant Funds		38,052.73
Income from Retirement Fund		603.30
Cash received from Sale of Plant Assets deposited in Current Cash (Motor-Boat)		50.00
		$158,839.32
Deduct:		
Payments from Current Funds during Year for Plant Assets as shown in Schedule IV,		
Buildings	$ 479.17	
Equipment	5,464.23	
Library Books, etc.	23,099.38	
	$29,042.78	
Purchase of Books from Balance of General Education Board Gift of $50,000.00 for Purchase of Books	5,708.20	
Payment of Pensions from Retirement Fund	720.00	
Excess of Expenses over Income for Year as shown in Exhibit B	3,767.25	39,238.23
Balance, December 31, 1930—Exhibit A		$119,401.09

Respectfully submitted,

LAWRASON RIGGS, JR.,
Treasurer.

V. THE REPORT OF THE LIBRARIAN

The important feature of 1930, which was the establishment of a regular endowment fund for the Library which would ordinarily give, along with the usual laboratory allowance, about $24,000 annually, was included in the report of last year. A general statement of the future apportionment of this sum as there given has been carried out in fact. A very important addition occurred in the spring, however, when the Director of the Woods Hole Oceanographic Institution placed $5,000 at the disposal of the Library to be used exclusively for the purchase of oceanographic books and journals. Of this amount, $2,149.73 had been spent at the end of the year 1930. The items thus purchased are indicated specifically in the general statement of additions to the Library as follows: journal subscriptions were 346, 24 new, and of these 5 were for the Woods Hole Oceanographic Institution. One hundred and fifteen books were purchased, 45 for oceanography. Back sets of journals were filled in complete to the number of 45, and 37 only partially completed—none of these were regarded as strictly for the Woods Hole Oceanographic Institution. The number of journals received in exchange for the Biological Bulletin was 442, an increase of 22, and 15 back sets that we needed were filled in. The reprint collection was augmented by 5,573.

The Library consists, then, of 26,519 bound journal volumes, 4,991 books, 64,231 reprints; and is receiving 1,060 current journals.

Gifts of books have been made to the Library by the following publishers, and the Librarian takes this opportunity to acknowledge these in the name of the Marine Biological Laboratory Library, although formal thanks have in all cases been directly addressed by letter.

P. Blakiston's Son & Co.	9
R. R. Bowker Co.	1
Chemical Foundation, Inc.	1
Chicago University Press	5
Harvard University Press	2
Paul B. Hoeber	2
Henry Holt & Co.	1
Alfred A. Knopf	2
J. B. Lippincott Co.	1
McGraw-Hill Book Co., Inc.	5
Macmillan Co.	21
C. V. Mosby Co.	1
W. B. Saunders Co.	4
Wm. Wood & Co.	2
Yale University Press	1

VI. THE REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I beg to submit herewith a report of the forty-third session of the Marine Biological Laboratory for the year 1930.

1. *Attendance.* The attendance for 1930 showed a slight increase over that of the preceding year in the numbers of both investigators and students, the figures for 1930 being 337 investigators and 136 students as compared with 329 investigators and 125 students in 1929. An inspection of the Tabular View of Attendance on page 28 will show that since 1927, when for the first time the research rooms in both the brick and the wooden buildings were practically all in use at the same time, the number of independent investigators has scarcely changed, except for the record-breaking summer of 1929 when visiting foreign physiologists, most of whom came to the Laboratory after the crowded season, swelled the total to figures not likely to be reached under normal conditions. On the other hand, investigators under instruction and research assistants whose numbers are not limited by that of the smaller laboratories have increased from 85 to 120 in the same period. The limit for the further increase of this class of investigators, however, is now in sight, and at the present rate will soon be reached.

The number of students, being limited by action of the Trustees, has shown only minor fluctuations for many years. The slight falling-off in 1929, caused chiefly by the change in that year in the times for holding the courses, was more than made up in 1930, though the maximum number which may at present be admitted to the courses, namely 142, has not yet been reached. This failure of the registration to reach its maximum value is not due to a deficiency of applications, since in nearly all of the courses the number of applicants greatly exceeds the number of available places, but rather to late withdrawals of students who have been accepted. To discourage such withdrawals, which are unfair to the rest of the applicants, who have usually in the meantime made other arrangements for the summer, the Executive Committee has recently voted to make a substantial increase in the registration fee which is forfeited in case of withdrawal.

Following the custom of the past three years, there are here presented figures which show the distribution of the attendance of investigators throughout the four seasons, including that of 1930, for which the necessary records have been kept.

		1927	1928	1929	1930
May	30	7	15	9	6
June	10	50	64	55	50
"	20	114	140	139	153
"	30	212	240	197	208
July	10	247	281	238	253
"	20	247	282	242	250
"	30	245	272	249	253
August	10	234	250	256	254
"	20	208	226	243	245
"	30	168	183	220	204
September	10	110	112	157	122
"	20	50	43	59	44
"	30	12	14	14	8

2. *The Report of the Treasurer.* This report shows that the total assets of the Laboratory at the end of 1930 were $2,660,559.11 as compared with $2,660,478.82 at the end of 1929. A further analysis of the figures shows that the book value of the endowment fund has remained practically stationary (though the Treasurer calls attention to a gratifying appreciation in the market value of the securities represented) while additions to the plant assets, chiefly in the form of books and new equipment, have about balanced the estimated depreciation on buildings and equipment. A decrease in the value of the land held by the Laboratory and the simultaneous appearance of a reserve fund of approximately twenty thousand dollars are accounted for by the sale by the Laboratory to the Woods Hole Oceanographic Institution of the tract of land upon which the new building of the latter institution now stands.

The income of the Laboratory increased from $200,408.91 in 1929 to $210,110.86 in 1930. A part of this increase is of a temporary nature only, as for example that from the sale of duplicate sets by the library; of the remainder the largest single item is the additional income from the funds appropriated last year by the General Education Board. In 1930 for the first time the full annual income from this fund became available. Since, however, this fund is at present being applied exclusively to the support of the library, the income available for general laboratory purposes remains practically unchanged.

The ordinary expenses of the Laboratory during 1930 showed a comparatively small increase over those for 1929, but expenses incidental to the reorganization of the Supply Department and the creation of a Museum, and, in particular, the reduction of the inventory of the Supply Department by discarding material originally valued at several thousand dollars, but for which there is at present little or no sale, have again prevented the appearance of a favorable balance, the excess of expenses over income after making allowance for depreciation being $3,767.25 as

compared with the similar deficit on paper of $855.33 for 1929. It is evident, however, when account is taken of the unusual expenses of the Supply Department in 1930 and of the necessarily large allowances for depreciation, that the finances of the Laboratory are in a very sound condition.

In 1930, for the first time in many years, the sum paid to the Laboratory for research space, chiefly by subscribing and coöperating institutions, showed a slight decrease. This was to have been expected in view of business conditions, which have materially reduced the incomes of most of the institutions concerned. It is a very encouraging fact, however, that the decrease even under these abnormal conditions amounted to less than three per cent.

3. *The Report of the Librarian* shows a continuation of the steady growth of the library which has been made possible especially by the generous support of the General Education Board. For purposes of comparison the figures for 1930 may be added to those listed in the Director's Report for 1929.

	1925	1926	1927	1928	1929	1930
Serials received currently	500	628	764	874	985	1060
Total number of bound volumes	15000	18200	22800	26500	28300	31500
Reprints	25000	38000	43000	51000	59000	64000

One especially noteworthy feature of the past year has been the strengthening of the part of the library devoted to the subject of oceanography, the development in this direction having been made possible by a cooperative arrangement with the Woods Hole Oceanographic Institution, assisted by special funds appropriated by that institution.

4. *Lectures and Scientific Meetings.* During the season of 1930 the number of formal scientific lectures, including the Reynold A. Spaeth Memorial Lecture delivered by Professor Hardolph Wastenys of the University of Toronto, was thirteen, with several other evenings devoted to non-scientific lectures and motion pictures. In addition, there were held 13 less formal meetings at which 56 shorter papers, whose titles are given on pages 31 to 33, were presented and discussed. Two of these meetings were of especial interest. The first, held on June 27, assumed the character of a celebration of the sixtieth birthday of Dr. Frank R. Lillie and of the fortieth consecutive year of his connection with the Marine Biological Laboratory. In addition to the scientific papers presented on that occason, which were all based upon work having its inception in Dr. Lillie's laboratory, a special address of congratulation was delivered by Dr. E. B. Wilson, and a ship's clock, the gift of Dr. Lillie's former students, was presented to him by Dr. L. V.

Heilbrunn. The evening was concluded by an informal reception at the M. B. L. Club. The second meeting of an unusual character was the one held on the morning of July 26 at which 12 papers in the field of neuro-muscular physiology were presented and discussed. This meeting formed the most important part of a two days' program, social as well as scientific in character, which was arranged by the workers in this field and was attended not only by laboratory workers but by a number of physiologists from a distance. So successful was this meeting that it is to be hoped that similar ones, devoted to various fields of biological research may be held in the future.

5. *Supply Department and Museum.* At the last annual meeting of the Board of Trustees it was voted to develop for the use of investigators and students working in Woods Hole a museum in which specimens of the local fauna and flora may at all times be available for purposes of study, and in which there may at the same time be preserved full records of the distribution of all the local forms, the seasons of their maximum abundance, their breeding habits, etc. The development of this important activity of the Laboratory was very appropriately placed in the hands of Mr. George M. Gray, whose long experience as Curator of the Supply Department has given him unique qualification for such a position. In order to fill the vacancy thus created in the Supply Department and to provide for a possible ultimate separation of the two present functions of this Department, namely, that of supplying living material for experimental purposes to workers at the Laboratory and of furnishing preserved material to schools and colleges, the General Biological Supply House of Chicago was invited to assume its temporary management. In preparation for the new arrangement, Dr. D. L. Gamble, representing this firm, spent several months in residence in Woods Hole during the summer of 1930 and has since continued the general supervision of this Department from Chicago with very satisfactory results, being ably assisted by Mr. James McInnis as Resident Manager.

6. *Facilities for Work with X-rays.* During the summer of 1930, through an appropriation of $500.00 by the Committee on the Effects of Radiation upon Living Organisms of the National Research Council and with the active assistance of the Chairman of that Committee, Professor W. C. Curtis, special facilities, not hitherto available for work with X-rays and other radiations were provided for workers at the Laboratory. In particular, there was made available throughout the summer the expert advice of Dr. G. Failla of the Memorial Hospital, New York, together with the assistance of competent technicians in the operation of the apparatus. Several manufacturers also furnished very valuable aid

of various sorts which is here gratefully acknowledged. So successful was this arrangement in 1930 that it is gratifying to be able to announce at the time of the writing of this report that it will be continued and somewhat extended in 1931.

7. *The Woods Hole Oceanographic Institution.* The position which Woods Hole has held for many years as one of the leading biological centers in the United States was materially strengthened by the erection during the past year of the large and splendidly equipped building which will be the permanent headquarters of the Woods Hole Oceanographic Institution. The land on which this building stands was formerly held by the Marine Biological Laboratory and leased by it to the Bar Neck Corporation. At a special meeting of the Board of Trustees, held in Washington, D. C., on April 28, 1930, it was voted to enter into an agreement with the Woods Hole Oceanographic Institution and the Bar Neck Corporation by which the former institution would acquire by purchase from the Marine Biological Laboratory approximately 54,000 square feet of the westerly portion of the so-called "Bar Neck Wharf." Full details as to this agreement will be found in the Report of the Auditors for 1930. Though there is no official connection between the Woods Hole Oceanographic Institution and the Marine Biological Laboratory, the work of each institution will supplement that of the other, and it is planned that there shall be close scientific coöperation between them. This coöperation has already assumed the form of a sharing of library facilities and Mess accommodations and will be extended in the future in all possible ways.

8. *The Board of Trustees.* One change in the Board of Trustees occurred during the past year, Professor William Patten of Dartmouth College having been elected Trustee Emeritus at the annual meeting of the Corporation and Professor E. R. Clark of the University of Pennsylvania having been selected to fill the place thereby made vacant in the Class of 1934.

9. *Gifts.* Appreciative acknowledgment is made of the gift by Mr. Ware Cattell and the "Collecting Net" of $500.00 for scholarships to students who in the courses given by the Laboratory show unusual promise as research workers.

There are appended as parts of this report:

1. The Staff, 1930.
2. Investigators and Students, 1930.
3. A Tabular View of Attendance, 1926–1930.
4. Subscribing and Coöperating Institutions, 1930.
5. Evening Lectures, 1930.

6. Shorter Scientific Papers, 1930.
7. Members of the Corporation, August, 1930.

Respectfully submitted,

M. H. JACOBS,
Director.

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1. THE STAFF, 1930

MERKEL H. JACOBS, *Director*, Professor of General Physiology, University of Pennsylvania.
Associate Director: ——

ZOÖLOGY

I. INVESTIGATION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.
E. G. CONKLIN, Professor of Zoölogy, Princeton University.
CASWELL GRAVE, Professor of Zoölogy, Washington University.
H. S. JENNINGS, Professor of Zoölogy, Johns Hopkins University.
FRANK R. LILLIE, Professor of Embryology, University of Chicago.
C. E. MCCLUNG, Professor of Zoölogy, University of Pennsylvania.
S. O. MAST, Professor of Zoölogy, Johns Hopkins University.
T. H. MORGAN, Director of the Biological Laboratory, California Institute of Technology.
G. H. PARKER, Professor of Zoölogy, Harvard University.
E. B. WILSON, Professor of Zoölogy, Columbia University.
LORANDE L. WOODRUFF, Professor of Protozoölogy, Yale University.

II. INSTRUCTION

J. A. DAWSON, Assistant Professor of Biology, College of the City of New York.
T. H. BISSONNETTE, Professor of Biology, Trinity College.
E. C. COLE, Associate Professor of Biology, Williams College.
O. E. NELSEN, Instructor in Zoölogy, University of Pennsylvania.
A. W. POLLISTER, Instructor in Zoölogy, Columbia University.
L. P. SAYLES, Instructor in Biology, College of the City of New York.
A. E. SEVERINGHAUS, Assistant Professor of Anatomy, College of Physicians and Surgeons, Columbia University.

JUNIOR INSTRUCTORS

B. R. COONFIELD, Professor of Biology, Southwestern College.
I. B. HANSEN, Graduate Student, University of Chicago.

PROTOZOÖLOGY

I. INVESTIGATION

(*See Zoölogy*)

II. INSTRUCTION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.
RACHEL BOWLING, Instructor in Zoölogy, Columbia University.
ROBERTS RUGH, Assistant in Zoölogy, Columbia University.

EMBRYOLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

Hubert B. Goodrich, Professor of Biology, Wesleyan University.
Benjamin H. Grave, Professor of Biology, De Pauw University.
Charles Packard, Assistant Professor of Zoölogy, Institute of Cancer Research, Columbia University.
Charles G. Rogers, Professor of Comparative Physiology, Oberlin College.
B. C. Twitty, Instructor in Zoölogy, Yale University.

PHYSIOLOGY

I. Investigation

Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Ralph S. Lillie, Professor of General Physiology, University of Chicago.
Albert P. Mathews, Professor of Biochemistry, University of Cincinnati.

II. Instruction

Teaching Staff

William R. Amberson, Assistant Professor of Physiology, University of Pennsylvania.
Philip Bard, Assistant Professor of Physiology, Princeton University.
Hallowell Davis, Assistant Professor of Physiology, Harvard University.
Ralph W. Gerard, Assistant Professor of Physiology, University of Chicago.
Charlotte Haywood, Assistant Professor of Physiology, Vassar College.
Leonor Michaelis, Member of the Rockefeller Institute, New York City.

Special Lecturers

Edwin J. Cohn, Associate Professor of Physical Chemistry, Harvard University.
Henry J. Fry, Associate Professor of Biology, Washington Square College, New York University.
E. Newton Harvey, Professor of Physiology, Princeton University.
Selig Hecht, Professor of Biophysics, Columbia University.
Merkel H. Jacobs, Professor of General Physiology, University of Pennsylvania.
Balduin Lucké, Associate Professor of Pathology, University of Pennsylvania.

BOTANY

I. Investigation

B. M. Duggar, Professor of Physiological and Economic Botany, University of Wisconsin.
C. E. Allen, Professor of Botany, University of Wisconsin.

S. C. Brooks, Professor of Zoölogy, University of California.
Ivey F. Lewis, Professor of Biology, University of Virginia.
Wm. J. Robbins, Professor of Botany, University of Missouri.

II. Instruction

William Randolph Taylor, Professor of Botany, University of Pennsylvania.
H. C. Bold, Instructor in Botany, University of Vermont.
James P. Poole, Professor of Evolution, Dartmouth College.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian.
Deborah Lawrence, Secretary.
Hester Ann Bradbury, Lillian F. Briggs, Mary A. Rohan, Assistants.

CHEMICAL SUPPLIES

Oliver S. Strong, Professor of Neurology and Neuro-Histology, Columbia University, Chemist.

APPARATUS ROOM

Samuel E. Pond, Assistant Professor of Physiology, Medical School, University of Pennsylvania, Custodian of Apparatus.

MUSEUM

George M. Gray, Curator.

SUPPLY DEPARTMENT

James McInnis, Manager.
A. M. Hilton, Collector.
Milton B. Gray, Collector.
Walter Kahler, Collector.
Geoffrey Lehy, Collector.
A. W. Leathers, Shipping.

BOATS

John J. Veeder, Captain.
E. M. Lewis, Chief Engineer.

F. M. MacNaught, Business Manager.
Herbert A. Hilton, Superintendent of Buildings and Grounds.
Thomas Larkin, Superintendent of Mechanical Department.
Lester F. Boss, Mechanician.
J. D. Graham, Glass-blowing Service.
A. R. Apgar, Photographic Service.
William Hemenway, Carpenter.

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2. INVESTIGATORS AND STUDENTS, 1930

Independent Investigators

Abramson, Harold A., Instructor, Harvard University.
Amberson, William R., Assistant Professor of Physiology, University of Pennsylvania.

ARMSTRONG, PHILIP B., Instructor in Anatomy, Cornell University Medical College.
ASHWORTH, JAMES H., Professor of Natural History, University of Edinburgh.
ASTROM, I. ELISABETH, Class Assistant, University of Toronto.
BAITSELL, GEORGE A., Professor of Biology, Yale University.
BAKER, HORACE B., Associate Professor, University of Pennsylvania.
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BARD, PHILIP, Assistant Professor of Physiology, Princeton University.
BARRON, E. S. GUZMAN, Instructor in Medicine, Johns Hopkins University Medical School.
BARTH, L. G., National Research Fellow, University of Chicago.
BEAMS, H. W., Dupont Fellow, University of Virginia.
BELKIN, MORRIS, Instructor, New York University.
BIDDLE, RUSSELL L., Teaching Fellow, California Institute of Technology.
BISSONNETTE, T. HUME, Professor of Biology, Trinity College.
BLANCHARD, KENNETH C., Associate Professor, New York University.
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BRONFENBRENNER, J., Professor of Bacteriology, Washington University Medical School.
BRONK, DETLEV W., Professor of Biophysics and Director of Johnson Foundation for Medical Physics, University of Pennsylvania.
BROOKS, MATILDA M., Research Associate in Biology, University of California.
BROOKS, S. C., Professor of Zoölogy, University of California.
BURDICK, DONALD L., Instructor in Biology, Union College.
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CAROTHERS, E. ELEANOR, Lecturer in Zoölogy, University of Pennsylvania.
CATTELL, WARE, Research Fellow in Biophysics, Memorial Hospital.
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CHRISTIE, JESSE R., Associate Nematologist, United States Department of Agriculture.
CLOWES, G. H. A., Director, The Lilly Research Laboratory, Eli Lilly & Co.
COBB, N. A., Agricultural Technologist and Nematologist, United States Department of Agriculture.
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COLE, ELBERT C., Associate Professor of Biology, Williams College.
COLE, KENNETH S., Assistant Professor of Physiology, Columbia University.
COONFIELD, BENJAMIN R., Professor of Biology, Southwestern College.
COOPER, GEORGE O., Instructor, University of Wisconsin.
COPELAND, MANTON, Professor of Biology, Bowdoin College.
COWLES, R. P., Associate Professor of Zoölogy, Johns Hopkins University.
CURTIS, W. C., Professor of Zoölogy, University of Missouri.
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FAILLA, G., Physicist, Memorial Hospital, New York.
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SCHMIDT, LEON H., University of Cincinnati.
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SCHRADER, FRANZ, Associate Professor, Bryn Mawr College.
SCHRADER, SALLY-HUGHES, Instructor in Biology, Bryn Mawr College.
SCHULTZ, JACK, Research Assistant, Carnegie Institution of Washington.
SEVERINGHAUS, AURA E., Associate in Anatomy, Medical School, Columbia University.
SHARMA, B. M., Professor of Anatomy, Tibbi Medical College.
SHOUP, CHARLES S., Assistant Professor of Biology, Vanderbilt University.
SHULL, A. FRANKLIN, Professor of Zoölogy, University of Michigan.
SHUMWAY, WALDO, Professor of Zoölogy, University of Illinois.
SICHEL, FERDINAND J., Assistant Instructor, Washington Square College, New York University.
SLIFER, ELEANOR H., Graduate Student, University of Pennsylvania.
SMITH, FREDERICK, Research Assistant, Rockefeller Institute.
STEINBACH, H. B., Graduate Student, Brown University.
STOCKARD, CHARLES R., Professor of Anatomy, Cornell University Medical College.
STRONG, OLIVER S., Professor of Neurology and Neuro-Histology, Columbia University.
STUNKARD, H. W., Professor of Biology, New York University.
STURDIVANT, HARWELL P., Instructor, Columbia University.
STURTEVANT, A. H., Professor of Genetics, California Institute of Technology.

SUMWALT, MARGARET, Assistant Professor, Woman's Medical College.
TASHIRO, SHIRO, Professor of Biochemistry, The University of Cincinnati.
TAYLOR, WM. RANDOLPH, Professor of Botany, University of Pennsylvania.
THOMPSON, J. W., Instructor, Swarthmore College.
TOWER, SARAH S., Instructor in Anatomy, Johns Hopkins University.
TWITTY, VICTOR C., Instructor in Zoölogy, Yale University.
UHLENHUTH, EDUARD, Professor of Gross Anatomy, University of Maryland Medical School.
VAN CLEAVE, C. D., Instructor in Anatomy, University of Pennsylvania.
VAN SLYKE, E., Instructor, University of Pittsburgh.
WANG, CHI CHE, 1314 East 56th Street, Chicago, Illinois.
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WHEDON, ARTHUR D., Professor of Zoölogy and Head of Department, North Dakota Agricultural College.
WHITAKER, D. M., Assistant Professor of Zoölogy, Columbia University.
WHITING, ANNA R., Professor, Head of Department of Biology, Pennsylvania College for Women.
WHITING, P. W., Associate Professor of Zoölogy, University of Pittsburgh.
WIEMAN, H. L., Professor of Zoölogy, University of Cincinnati.
WILLIER, B. H., Associate Professor of Zoölogy, University of Chicago.
WILSON, EDMUND B., DaCosta Professor of Zoölogy, Emeritus, Columbia University.
WILSON, F. EDWARD, Graduate Student, Clark University.
WILSON, MAY G., Associate, Department of Pediatrics, Cornell University Medical College.
WOLF, E. ALFRED, Assistant Professor of Zoölogy, University of Pittsburgh.
WOODRUFF, L. L., Professor of Protozoölogy, Yale University.
WOODS, FARRIS H., Assistant Professor of Zoölogy, University of Missouri.
YOUNG, WILLIAM C., Instructor in Biology, Brown University.
ZELENY, CHARLES, Professor of Zoölogy, University of Illinois.

Beginning Investigators

BAILEY, SARAH W., Graduate Student, Radcliffe College.
BROWN, FRANK A., JR., Austin Teaching Fellow, Harvard University.
CALDWELL, LUCILE, Johns Hopkins University.
CHANG, J. H., Graduate Student, University of Chicago.
CHEN, H. T., Student, Harvard University Medical School.
CLINE, ELSIE, Graduate Student, The Johns Hopkins University.
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CURTIS, MARY ELIZABETH, Graduate Student, Yale University.
DAMBACH, GEORGE J., Graduate Assistant, University of Pittsburgh.
DEARING, WILLIAM H., Graduate Student, University of Pennsylvania.
DEITRICH, JOHN E., Medical Student, Johns Hopkins University.
DIXON, EVELYN C., Graduate Student, Washington University.
DRAPER, JOHN W., Cornell University Medical College.
DREW, WILLIAM, Massachusetts Agricultural College.
DUSHANE, GRAHAM, Wabash College.
ETKIN, WILLIAM, Tutor, College of the City of New York.
FLAMMON, SISTER M. MURIEL, Instructor in Biology, Seton Hill College.
FRENCH, C. S., Harvard University.
GEBRAK, ANTON, Moscow Agricultural Academy.

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KINSBERGEN, MAURICE, Assistant, New York University.
KLOSE, THEODORA, Instructor in Botany, Vassar College.
LEE, KATY, Graduate Assistant in Zoölogy, University of Missouri.
LHÉRISSON, CAMILLE, Professor of Biology, University of Haiti Medical School.
LORBERBLATT, ISAAC, Student, Washington University Medical School.
MCGOUN, RALPH C., JR., Instructor in Biology, Amherst College.
MACMURRAY, MARY T., 8629 109th Street, Richmond Hill, New York.
MACKMULL, GULDEN, Demonstrator of Histology and Embryology, Baugh Institute of Anatomy.
MILLER, EVELYN H., Graduate Student, University of Pennsylvania.
MILLER, FORREST W., Graduate Assistant, University of Pittsburgh.
MONAGHAN, BETTY R., Assistant, Washington University.
MORRIS, SAMUEL, Instructor in Zoölogy, University of Pennsylvania.
NELSON, PHYLLIS M., Washington University.
PARPART, ARTHUR K., Instructor and Graduate Student, University of Pennsylvania.
PARPART, ETHEL R., Technician, University of Pennsylvania.
PITTS, ROBERT F., Student Assistant, Johns Hopkins University.
RANKIN, DOUGLAS, Johns Hopkins University.
ROBERT, NAN L., Instructor, Hunter College.
SANTOS, FELIX V., Graduate Student, The University of Chicago.
SAVIN, MARION B., Graduate Student, University of Pennsylvania.
SCARBOROUGH, J. ELLIOTT, JR., Student of Medicine, Harvard University.
SCHECHTER, VICTOR, Tutor in the Department of Biology, College of the City of New York.
SCHWEITZER, MORTON D., Assistant in Zoölogy, Columbia University.
SCOTT, SISTER FLORENCE M., Assistant Professor of Biology, Seton Hill College.
SHAPIRO, HERBERT, Assistant in Zoölogy, Columbia University.
SHAW, C. RUTH, Graduate Assistant, University of Pittsburgh.
SKINNER, B. F., Graduate Student, Harvard University.
SMITH, SUZANNE G., Graduate Assistant in Zoölogy, University of Missouri.
SMYTHE, C. V., Fellow National Research Council, Rockefeller Institute.
SONNEBORN, TRACY M., Research Assistant, Johns Hopkins University.
STABLER, ROBERT M., Assistant Instructor, University of Pennsylvania.
STANCATI, MILTON F., Graduate Assistant, University of Pittsburgh.
STEWART, DOROTHY R., Assistant Professor of Biology, Skidmore College.

STEINER, MATTHEW M., Assistant in Biology, New York University.
STREET, SIBYL F., Assistant to Department of Zoölogy, Vassar College.
STUCK, FLORENCE, Student, Columbia University.
TANG, PEI-SUNG, Johns Hopkins University.
TEWINKEL, LOIS E., Assistant in Zoölogy, Barnard College, Columbia University.
TOOTHILL, MARTHA C., Assistant in Biology, Brown University.
TUAN, HSU-CHUAN, Graduate Student, University of Pennsylvania.
TURNER, CLARENCE D., Research Assistant, University of Missouri.
WATERS, NELSON F., Research Assistant in Applied Botany, Harvard University.
WELLS, EVELYN, Instructor in Biology, St. Mary's Seminary.
WELLS, L. J., Graduate Student, University of Chicago.
WILDE, MARY H., Graduate Assistant, New Jersey College for Women.
WRIGHT, CHARLES I., Fellow in Physiology, University of Rochester Medical School.
YANCEY, PATRICK H., Graduate Student, St. Louis University.

Research Assistants

BECK, L. V., Teaching Fellow, New York University.
BERNSTEIN, ALAN, Research Assistant, New York University.
BUCK, LOUISE H., Research Assistant, New York University.
CAMPBELL, RAYMOND W., Assistant, Fatigue Laboratory, Harvard University.
DAVIDSON, SYDNEY A., Williams College.
EISENBRANDT, W. H., Student, University of Maryland Medical School.
FRANCIS, DOROTHY S., Research Assistant, Memorial Hospital.
FRIEDMAN, HILDA, Assistant in Pathology, Washington University Medical School.
FRIEDHEIM, ERNST A. H., Rockefeller Institute.
GRAND, CONSTANTINE G., Research Assistant, New York University.
GREENBERG, JACOB, Medical Student, Yale University Medical School.
HARRYMAN, ILENE, Research Assistant, Lilly Research Laboratory.
HEUSNER, A. P., Student, Swarthmore College.
HOFFMAN, OLIVE D., Research Assistant, New York University.
LAZAROVICH-HREBELIANOVICH, MARA DE, Research Assistant, New York University.
MENDELSON, E. S., Research Assistant, University of Pennsylvania.
MENKIN, MIRIAM F., Henry Phipps Institute.
OBERG, S. ALBERT, Harvard University.
PARKS, ELIZABETH K., Graduate Assistant in Zoölogy, Oberlin College.
RAVENSWAAY, VAN A. C., Research Assistant, Washington University.
REYNOLDS, SARA JANE, Research Assistant, New York University.
RUDNICK, DOROTHEA, Research Assistant, University of Chicago.
SALOMON, KURT, Fellow of the Rockefeller Foundation, Rockefeller Institute for Medical Research.
SANDERS, ROSALTHA, Technician, Yale University.
SCHUBERT, MAXWELL, Assistant, Rockefeller Institute for Medical Research.
SCOTT, ALLAN C., Graduate Assistant, University of Pittsburgh.
SHATTUCK, G. EDGAR, Assistant Instructor in Physiology, University of Pennsylvania.
SHEAR, M. J., Administrative Officer and Research Chemist, Pediatric Research Laboratory, Jewish Hospital.
SHLAER, SIMON, Research Assistant, Columbia University.
SMITH, M. DOREEN, Research Assistant, Memorial Hospital.
SWANN, SHERLOCK, Research Associate, University of Illinois.
TOCKER, ALBERT M., Student, Washington University Medical School.
WALLACE, EDITH M., Artist and Research Assistant, Carnegie Institution of Washington.

Students

BOTANY

Breed, Helen L., Student, Wellesley College.
Brunel, Jules, Assistant Professor of Botany, University of Montreal.
Cheever, Clarence A., Boston Society of Natural History.
Drouet, Francis, Graduate Assistant, University of Missouri.
Forbes, John M., Student, Harvard University.
Glidden, Dorothy P., Student, Smith College.
Hopkins, Milton, Student, Amherst College.
Huntington, Evelyn, Student, Vassar College.
Klose, Theodore G., Instructor in Botany, Vassar College.
Loughridge, Gasper A., Laboratory Assistant in Botany, Rutgers University.
McKee, Jewel C., University of Wisconsin.
Oppenheimer, Jane M., Student, Bryn Mawr College.
Roland, Albert E., Student, Acadia University.
Safford, Decius W., Dartmouth College.
Stewart, Paul A., Student, University of Rochester.
Wilde, Mary H., Assistant, New Jersey College for Women.

EMBRYOLOGY

Baker, E. G. Stanley, Student, DePauw University.
Ballard, Overton T., University of Illinois.
Bamber, Lyle E., Graduate Student and Assistant, University of Illinois.
Carter, George H., Student, Amherst College.
Dawson, Helen L., Graduate Student, Washington University.
Derbyshire, Arthur J., Jr., Harvard University.
Earl, Ruth R., Technician, College of the City of New York.
Eaton, Theodore H., Jr., Cornell University.
Green, David E., Assistant, Washington Square College.
Guerlac, Henry E., Student, Cornell University.
Hileman, Clara M., Instructor in Biology, Penn State College.
Hjortland, Arthur L., Assistant, University of Illinois.
Hunninen, Arne V., Student, Wesleyan University.
Johnson, Arlene C., Student, Wheaton College.
Johnson, Myra L., Student, Smith College.
Lane, Mary, Smith College.
Loefer, John B., Graduate Assistant in Biology, New York University.
Mackmull, Gulden, Demonstrator of Histology and Embryology, Baugh Institute of Anatomy, Jefferson Medical College.
Maxwell, Florence J., Instructor, Carnegie Institute of Technology.
Nichols, Rowena, Wellesley College.
Patch, Esther M., Teacher of Biology, East Boston High School.
Reid, Marion A., Instructor, Boston University Medical School.
Riley, Lena C., Student, Wellesley College.
Rosenbaum, Louise, Student, University of Pennsylvania.
Scott, John P., University of Wyoming.
Stanley, Willard F., Graduate Student and Research Assistant, University of Illinois.
Wells, Lemen J., Graduate Student, University of Chicago.

PHYSIOLOGY

Appelrot, Samuel, Fellow, Rockefeller Foundation.
Behner, Dorothy M., Assistant, New York University.
Chang, Tsung H., Graduate Student, University of Chicag .

CHEN, TUANG T., Assistant in Biochemistry, Peking Union Medical College.
DANN, MARGARET, Assistant in Physiology, Cornell University Medical College.
DREW, WILLIAM B., Massachusetts Agricultural College.
DUBOIS, DELAFIELD, New York University.
FENG, TE-PEI, Graduate Student, University of Chicago.
GARDNER, EDITH MCN., Assistant in Physiology, Vassar College.
GATES, FREDERICK L., Research Fellow, Harvard University.
HEGNAUER, ALBERT H., Fellow in Physiology, University of Rochester Medical School.
LEITCH, JAMES L., University of California.
MONAGHAN, BETTY R., Assistant and Graduate Student, Washington University.
OLIPHANT, JOSEPH F., Instructor in Biology, Union College.
OSTER, ROBERT H., Student, Williams College.
PITTS, ROBERT F., Johns Hopkins University.
SHAW, GRETCHEN, Graduate Student, University of Chicago.
STEINER, MATTHEW M., Assistant in Biology, New York University.
STEVENS, THELMA O., Graduate Assistant, Mt. Holyoke College.
TANG, PEI-SUNG, Harvard University.
VACK, CHRISTINE M., Technician, Harvard University Medical School.
VICARI, EMILIA M., Research Associate, Cornell University Medical College.
WOODWARD, ALVALYN E., Assistant Professor, University of Michigan.

PROTOZOÖLOGY

BREHME, KATHERINE S., Barnard College.
CARTER, HELEN D., Elmira College.
COSTELLO, DONALD P., 3732 Heidelberg, Detroit, Michigan.
EMBICH, JOHN R., Graduate Student, Columbia University.
FLAMMON, SISTER M. MURIEL, Seton Hill College.
FRYE, MARY ELIZABETH, Pennsylvania College for Women.
LHÉRISSON, CAMILLE, Professor of Biology, University of Haiti Medical School.
MASTEN, LOIS E., Elmira College.
MORGAN, WILLIE A., Assistant Instructor in Biology, Coker College.
SCHOELT, ABRAHAM H., Graduate Student, Columbia University.
SCOTLAND, MINNIE B., Teacher, New York State College for Teachers.
SMITH, CLAIRE M., Hunter College.
STEINBERG, BERNHARD, Director of Laboratories and Research, Toledo Hospital.
WEISMAN, MAXWELL N., Fellow, College of the City of New York.

INVERTEBRATE ZOÖLOGY

ALDERMAN, EVANGELINE, Oberlin College.
BAUMGARTNER, FREDERICK M., Butler University.
BITTINGER, ISABEL, Radcliffe College.
BOARDMAN, EDWARD T., Graduate Assistant, Johns Hopkins University.
BROWN, FRANK A., JR., Harvard University.
CAMPBELL, DAN H., Student, Wabash College.
CARLSON, J. GORDON, Assistant in Zoölogy, University of Pennsylvania.
CHADWICK, CLAUDE S., Instructor in Biology, Vanderbilt University.
COHEN, ROSE S., Graduate Assistant, University of Cincinnati.
COLEMAN, LUCILLE, Agnes Scott College.
COPLAN, HELEN M., Student, Goucher College.
CRAIG, ROBERT L., Student, Amherst College.
CROWELL, PRINCE S., JR., Bowdoin College.
DEE, M. BARBARA, Assistant in Science, Jamaica Plain High School.
DEROO, GRACE, Radcliffe College.
DERRICKSON, MARY B., Goucher College.

DORRIS, FRANCES S., Graduate Student, Yale University.
DOYLE, WILLIAM L., Johns Hopkins University.
EICHOLD, EVA C., Student, Newcomb College.
EVERETT, JOHN W., Yale University.
FARBER, SEYMOUR M., University of Buffalo.
FENNELL, R. A., Graduate Student, Duke University.
FISHER, KENNETH C., Student and Assistant, Acadia University.
FRENCH, CHARLES S., Harvard University.
HAMBURGER, LOUIS P., JR., Johns Hopkins University.
HART, HELEN B., Student, Wellesley College.
HASTINGS, MARGARET, Student, Mt. Holyoke College.
HAYES, FREDERICK R., Assistant Professor of Zoölogy, Dalhousie University.
HEISS, ELIZABETH M., Assistant in Biology and Histology, Purdue University.
HEUSNER, ALBERT P., Swarthmore College.
HEWITT, DOROTHY C., Graduate Student, Yale University.
HOLLOWAY, MAY P., Teacher of Science, Burke School.
HUBBARD, RUTH A., Assistant, Cleveland Museum of Natural History.
JACKSON, JEANNETTE A., Graduate Assistant in Zoölogy, Syracuse University.
JOHNSON, DOROTHY F., Laboratory Assistant, Wellesley College.
KILLE, FRANK R., Assistant Professor of Biology, Birmingham-Southern College.
KROC, ROBERT L., Graduate Assistant, University of Wisconsin.
LEAVITT, BENJAMIN B., Instructor in Zoölogy, Dartmouth College.
MENZEL, ARTHUR E. O., National Tuberculosis Research Fellow, Presbyterian Hospital.
MERRIMAN, DANIEL, Student, Harvard University.
MORRIS, SAMUEL, Instructor in Zoölogy, University of Pennsylvania.
PREST, MARGARET R., Graduate Assistant, Mt. Holyoke College.
REDMOND, ALBERT C., Student, Hamilton College.
REYNOLDS, ALBERT E., Assistant in Zoölogy, DePauw University.
RITTER, M. ESTHER, Student, Wilson College.
SHEA, MARGARET, Student, Oberlin College.
SIDEBOTHAM, RUTH S., Graduate Assistant in Zoölogy, Washington University.
SNELL, PETER A., Fellow in Biology, Princeton University.
SNOOK, THEODORE, Graduate Assistant in Zoölogy, Rutgers University.
SWANSON, OSCAR E., Student, Antioch College.
TIPTON, SAMUEL R., Graduate Student, Duke University.
TOWNSEND, GRACE, Instructor, Joliet Township High School and Junior College.
TREAT, DOROTHY A., Assistant in Department of Education, Cleveland Museum of Natural History.
WATERS, NELSON F., Graduate Student, Harvard University.
WEED, MILTON R., Student, Wesleyan University.
WOODRUFF, BETH H., Graduate Assistant, Western Reserve University.

3. TABULAR VIEW OF ATTENDANCE

	1926	1927	1928	1929	1930
INVESTIGATORS—Total	252	294	323	329	337
Independent	156	209	217	234	217
Under Instruction	84	57	81	71	87
Research Assistants	12	28	25	24	33
STUDENTS—Total	141	141	133	125	136
Zoölogy	56	57	57	53	56
Protozoölogy	19	17	16	15	14
Embryology	28	32	29	28	27
Physiology	18	19	15	17	23
Botany	20	16	16	12	16
TOTAL ATTENDANCE	393	435	456	454	473
Less Persons registered as both students and investigators	8	1	2	10	14
	385	434	454	444	459
INSTITUTIONS REPRESENTED—Total	119	111	111	123	126
By Investigators	84	89	80	96	95
By Students	60	63	66	64	71
SCHOOLS AND ACADEMIES REPRESENTED					
By Investigators	–	1	1	–	–
By Students	4	4	1	1	4
FOREIGN INSTITUTIONS REPRESENTED					
By Investigators	17	15	13	30	7
By Students	3	8	8	3	2

4. SUBSCRIBING AND COÖPERATING INSTITUTIONS

Acadia University
Amherst College
Antioch College
Beaver College
Bowdoin College
Brown University
Bryn Mawr College
Butler College
C. R. B. Educational Foundation
California Institute of Technology
Carnegie Institution of Washington
Chinese Educational Mission
Columbia University
Cornell University
Cornell University Medical College
Dalhousie University
Dartmouth College
DePauw University
Duke University
Elmira College
General Education Board
Goucher College
Hamilton College
Harvard University
Harvard University Medical School
Howard University
Hunter College
Indiana University
Industrial & Engineering Chemistry, of the American Chemical Society
Johns Hopkins University
Johns Hopkins University Medical School
Eli Lilly & Co.
Memorial Hospital of N. Y. City
Morehouse College
Mount Holyoke College
National Research Council
New York State Department of Health
New York University
Oberlin College

Pennsylvania College for Women
Princeton University
Radcliffe College
Rockefeller Foundation
Rockefeller Institute for Medical Research
Rutgers University
Seton Hill College
Smith College
Sophie Newcomb College
Southwestern
St. Louis University
Swarthmore College
Tufts College
Union College
United States Dept. of Agriculture
University of Buffalo
University of Chicago
University of Cincinnati
University of Illinois
University of Michigan
University of Missouri
University of Nebraska
University of Pennsylvania
University of Pennsylvania Medical School
University of Pittsburgh
University of Rochester
University of Tennessee
University of Virginia
University of Wisconsin
Vanderbilt University Medical School
Vassar College
Wabash College
Washington University
Washington University Medical School
Wellesley College
Wesleyan University
Western Reserve University
West Virginia State College
West Virginia University
Wheaton College
Wistar Institute of Anatomy and Biology
Yale University

SCHOLARSHIP TABLES

Lucretia Crocker Scholarships for Teachers in Boston.
Scholarship of $100 supported by a friend of the Laboratory since 1898.
The Edwin S. Linton Memorial Scholarship of Washington and Jefferson College.
The Bio Club Scholarship of the College of the City of New York.
Ida H. Hyde Scholarship of the University of Kansas.

5. EVENING LECTURES, 1930

Tuesday, July 1
DR. W. B. SCOTT ..... "New Light on the Development and Migrations of American Mammals."

Tuesday, July 8
DR. HENRY B. BIGELOW ..... "Prospects and Problems of Oceanography."

Tuesday, July 15
DR. H. S. JENNINGS ..... "Heredity and Mutation in Relation to Environment in Protozoa."

Friday, July 18
DR. EDWARD MELLANBY ..... "Food Deficiencies."

Tuesday, July 22
DR. F. R. LILLIE ..... "The Action of the Sex Hormones in the Fowl: An Account of the Chicago Experiments."

Tuesday, July 29
DR. LEONOR MICHAELIS ..... "The Reversible Oxidizable-reducible Systems Found in Living Organisms."

Tuesday, August 5
DR. E. F. DUBOIS ..... "Recent Progress in the Field of Respiratory Metabolism."

Tuesday, August 12
DR. E. CHATTON ..... "A Study of the Dinoflagellate, Polykrikos Schwartzi as a Basis for the Discussion of Some Problems of General Cytology."

Tuesday, August 19
THE REYNOLD A. SPAETH MEMORIAL LECTURE, delivered by DR. HARDOLPH WASTENEYS ..... "Protein Synthesis."

Thursday, August 21
DR. H. H. GRAN ..... "The Productivity of the Ocean."

Tuesday, August 26
DR. D. W. BRONK ..... "Nerve Impulse Rhythms and the Control of Movement."

Tuesday, September 2
DR. G. C. HIRSCH ..... "The Problem of Restitution with Special Regard to the Phenomena of Secretion."

Tuesday, September 9
DR. G. E. HUTCHINSON ..... "The Hydrobiology of Arid and Semi-arid Regions."

SPECIAL LECTURES AND MOTION PICTURES

Monday, August 11
"The Illyria Expedition to the Galapagos, the South Sea Islands, the New Hebrides, the Solomon Islands, New Guinea, Bali and Angkor." Exhibited by MR. CORNELIUS CRANE, the Leader of the Expedition.

Thursday, August 14
"The Florida Everglades and the Proposed Tropic Everglades National Park." Illustrated with colored lantern slides. MR. ERNEST F. COE, Chairman of the Tropic Everglades Park Association.

Thursday, August 28
Motion pictures.
"William Harvey and the Circulation of the Blood." Arranged by SIR THOMAS LEWIS.
"The Early Development of the Rabbit Egg." DR. W. H. LEWIS and DR. P. W. GREGORY.
"The Life Cycle of the Oyster." Prepared by the AMERICAN MUSEUM OF NATURAL HISTORY.

Friday, August 29
"Motion Pictures of Marine and Fresh Water Protozoa of the Woods Hole Region," RUTH B. HOWLAND, Department of Biology, New York University.

6. SHORTER SCIENTIFIC PAPERS, 1930

Tuesday, June 24

Dr. Balduin Lucké and Dr. Morton McCutcheon ..... "The Effect of Injury on Cellular Permeability to Water."

Dr. M. M. Brooks ..... "The Relation between rH and the Penetration of Oxidation-reduction Indicators."

Dr. S. C. Brooks ..... "Accumulation of Ions in Living Cells."

Dr. M. H. Jacobs, Mr. A. K. Parpart, Dr. W. A. Smith and Mr. G. E. Shattuck ..... "The Permeability of the Erythrocyte to Urea."

Friday, June 27

Dr. B. H. Willier ..... "The Developmental Relations of the Heart and the Liver in Chorio-allantoic Grafts."

Dr. E. E. Just ..... "Cortical Protoplasm and Vital Phenomena."

Dr. William C. Young ..... "The Post-testicular History of Spermatozoa and Reproduction in the Male Guinea Pig."

Dr. L. V. Heilbrunn ..... "The Action of Ultra-violet Rays on Arbacia Egg Protoplasm."

Thursday, July 3

Dr. Walter S. Root and Dr. Charlotte Haywood ..... "The Effect of Carbon Dioxide upon the Rate of Oxygen Consumption and of Cleavage of the Arbacia Egg."

Dr. L. Michaelis and Dr. K. Salomon ..... "Respiration of Erythrocytes."

Dr. R. W. Gerard ..... "Observations on the Metabolism of the Coccus, Sarcina lutea."

Dr. E. S. G. Barron ..... "The Effect of Methylene Blue upon the Respiration of Normal and of Cancer Tissue."

Friday, July 11

Dr. W. R. Taylor ..... "Chromosome Structure in Meiosis of Gasteria."

Dr. B. M. Duggar ..... "New Technique and Some Adsorption Studies with Virus Diseases of Plants."

Thursday, July 17

Dr. Vera Koehring ..... "Some Cytological Relationships in Narcosis."

Dr. Eleanor H. Slifer ..... "The Mitotic Activity in the Developing Grasshopper Egg."

Dr. A. F. Huettner ..... "Spermatogenesis in Drosophila melanogaster."
Dr. Hope Hibbard ..... "Cytological Studies on the Silk Gland of Bombyx mori."

Friday, July 25

Dr. S. Gelfan ..... "The All-or-None Law in Muscle."
Dr. W. R. Amberson, Mr. A. K. Parpart and Miss Gertrude Sanders ..... "Low Voltage Elements of the Action Potential Wave of Nerve."
Dr. F. O. Schmitt ..... "The Effect of Cyanides and Carbon Monoxide on Nerve."

Saturday, July 26

Dr. M. Dubuisson ..... "Cardiac Automatism in Invertebrates."
Dr. W. E. Garrey ..... "Observations on the Heart of Limulus."
Dr. D. J. Edwards ..... "The Action of Pressure on Some Physiological Processes."
Dr. G. H. Bishop ..... "The Influence of Iodo-acetic Acid on Muscle Contracture."
Dr. F. H. Pratt ..... "Experiments on the Terminal Nerve-muscle Unit."
Dr. D. W. Bronk ..... "Graded Muscular Contractions."
Dr. R. W. Gerard ..... "Nerve Metabolism and Asphyxia."
Dr. R. S. Lillie ..... "Recovery in the Passive Iron Wire Model."
Dr. A. M. Monnier ..... "Mathematical Analysis Applied to the Functions of the Nervous System."
Dr. H. Davis ..... "Re-education and Modification of Reflexes."
Dr. G. P. McCouch ..... "Patterns of Some Extra-ocular Reflexes in the Cat."
Dr. P. Bard ..... "The Behavior of a Cat without the Telencephalon."

Friday, August 1

Dr. Paul S. Henshaw ..... "Some Biological Effects Produced by Alpha Particles on Drosophila Eggs."
Mr. Ware Cattell ..... "The Effect of X-Rays upon the Growth of the Wheat Seedling."
Dr. W. C. Curtis ..... "Effects of X-Rays upon Regeneration."
Dr. Charles Packard ..... "The Relation between Division Rate and Susceptibility to Radiation."

Friday, August 8

Dr. Helen M. Miller ..... "Life Cycle of a Bisexual Rotifer."
Dr. Tracy M. Sonneborn ..... "Cause, Inheritance, and Effects of the Chain-forming Tendency in the Ciliate Protozoan, Colpidium."

Dr. Ruth Stocking Lynch ..... "The Effects of Long-continued Starvation in a Rotifer in Relation to Inheritance."

Dr. H. W. Stunkard ..... "The Life History of Cryptocotyle lingua."

Friday, August 15

Dr. Mary S. MacDougall ..... "A Mutation in Chilodon uncinatus Produced by Ultra-violet Radiation —A Preliminary Report."

Dr. R. D. Manwell ..... "The Effect of Quinine and Plasmoquin on Avian Malaria."

Dr. E. Chatton ..... "The Asymmetric Motile Stages of Epistylis and the Question of the So-called Longitudinal Division of the Vorticellidae."

Dr. Ruth B. Howland ..... "Cine-photomicrograph of Microinjection of Vacuolated Protoplasm."

Friday, August 22

Dr. Calvin B. Bridges ..... "The Neutralization of the Effects of Deficiencies through Duplications of the Same Chromosome Material."

Dr. A. H. Sturtevant ..... "A Peculiar Sex-ratio in Drosophila obscura."

Dr. Helen Redfield ..... "Studies of Crossing-over in Drosophila."

Dr. Jack Schultz ..... "The Eye of Pigments of Drosophila."

Friday, August 29

Dr. H. B. Goodrich and
Mr. I. B. Hansen ..... "Embryonic Development of Mendelian Characters in the Goldfish."

Dr. H. A. Abramson ..... "The Isoelectric Point of Mammalian Red Blood Cells."

Dr. E. N. Harvey and
Mr. A. L. Loomis ..... "The Microscope-centrifuge."

Friday, September 5

Dr. J. M. Johlin ..... "The Extraction of Micro-organisms."

Dr. W. E. Garrey and
Dr. W. R. Bryan ..... "Alkalosis in Relation to Tetany following High Temperatures after Parathyroidectomy."

Dr. K. Blanchard ..... "Catalysis of Condensation Reactions by Amino-acids."

Dr. L. Michaelis and
Dr. M. Schubert ..... "Metal Complex Compounds of Thioglycollic Acid."

7. MEMBERS OF THE CORPORATION

1. Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Md.
Billings, Mr. R. C., 66 Franklin St., Boston, Mass.
Conklin, Prof. Edwin G., Princeton University, Princeton, N. J.
Coolidge, Mr. C. A., Ames Building, Boston, Mass.
Crane, Mr. C. R., New York City.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.
Fay, Miss S. B., 88 Mt. Vernon St., Boston, Mass.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.
Gardiner, Mrs. E. G., Woods Hole, Mass.
Jackson, Miss M. C., 88 Marlboro St., Boston, Mass.
Jackson, Mr. Chas. C., 24 Congress St., Boston, Mass.
Kidder, Mr. Nathaniel T., Milton, Mass.
King, Mr. Chas. A.
Lee, Mrs. Frederic S., 279 Madison Ave., New York City, N. Y.
Lowell, Mr. A. Lawrence, 17 Quincy St., Cambridge, Mass.
Means, Dr. James Howard, 15 Chestnut St., Boston, Mass.
Merriman, Mrs. Daniel, 73 Bay State Road, Boston, Mass.
Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York City, N. Y.
Morgan, Prof. T. H., Director of Biological Laboratory, California Institute of Technology, Pasadena, Calif.
Morgan, Mrs. T. H., Pasadena, Calif.
Noyes, Miss Eva J.
Osborn, Prof. Henry F., American Museum of Natural History, New York, N. Y.
Phillips, Mrs. John C., Windy Knob, Wenham, Mass.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pa.
Sears, Dr. Henry F., 86 Beacon St., Boston, Mass.
Shedd, Mr. E. A.
Thorndike, Dr. Edward L., Teachers College, Columbia University, New York City, N. Y.
Trelease, Prof. William, University of Illinois, Urbana, Ill.
Ware, Miss Mary L., 41 Brimmer St., Boston, Mass.
Williams, Mrs. Anna P., 505 Beacon St., Boston, Mass.
Wilson, Dr. E. B., Columbia University, New York City, N. Y.

2. Regular Members, August, 1930

Adams, Dr. A. Elizabeth, Mount Holyoke College, South Hadley, Mass.

Addison, Dr. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pa.

Adolph, Dr. Edward F., University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

Allee, Dr. W. C., University of Chicago, Chicago, Ill.

Allen, Prof. Charles E., University of Wisconsin, Madison, Wis.

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THE RELATION BETWEEN CLEAVAGE AND TOTAL ACTIVATION IN ARTIFICIALLY ACTIVATED EGGS OF *URECHIS*

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It is generally assumed in most work on artificial parthenogenesis that cleavage and development result when the initial response of the egg to the artificial agent most closely resembles its response to the sperm. The percentage of eggs that respond in this fashion varies, of course, with the length of exposure to the artificial agent, presumably reaching a maximum for the exposure producing the highest percentage of activation.[1]

It would follow then that the cleavage-activation relation should be such that as the percentage of activation increases, the percentage of cleavage increases; that is, that the percentage of cleavage is directly proportional to the percentage of activation. Although this relation is practically always tacitly assumed in parthenogenesis experiments, detailed data on this point are lacking. If, however, exposures giving higher percentages of activation do not produce increasing percentages of eggs whose response is most nearly like that induced by the sperm, or if such eggs were not the ones which cleave and develop, an entirely different cleavage-activation relation might be expected. The determination of this relation is important, then, in an analysis of the factors which determine whether or not an artificially activated egg will cleave.

The variation of the percentage of activation with the length of exposure to the artificial agent is in itself a highly interesting fact, since it is not manifested in insemination of a normal batch of eggs with normal sperm. This variation may be attributed to variability in the amount of treatment necessary to activate a given egg, or, less likely perhaps, to a variation in the time at which the change produced by the activating agent reaches a given egg. Whatever its source, the way in which the percentage of activation varies with the length of exposure is useful in helping to elucidate the mechanism by which the artificial agent activates the egg.

[1] Any egg in which initial developmental changes have taken place will be termed "activated" in this paper, regardless of maturation or cleavage.

In the parthenogenesis experiments on *Urechis* eggs a unique relation between cleavage and activation was found, such that as the percentage of activation increases, the percentage of cleavage decreases. The variation of percentage activation with length of exposure was found to give a particular type of distribution curve in certain of the experiments. These results together with their interpretation are presented in detail in this report.

Material and Method

The eggs used in these experiments were those of the echiuroid, *Urechis caupo*, described by Fisher and MacGinitie (1928). The changes undergone by the egg upon normal fertilization, and upon artificial activation, and the method used in activating the eggs were described in detail in a previous publication (Tyler, 1931). Briefly, it was found that dilutions of sea water ranging from 80 per cent to distilled water were effective in activating the *Urechis* eggs.[2] In order to treat the eggs, a batch was transferred with as little sea water as possible to a Stender dish containing a large volume of the hypotonic solution. Samples were then removed after various intervals of time to Syracuse dishes containing normal sea water. All the usual precautions in regard to contamination by sperm or foreign matter, hypertonicity, etc., were taken.

Table I

Unfertilized Eggs Treated with Distilled Water, Temperature 21.8° C.

Length of Exposure	Activation	Cleavage of Activated Eggs
min.	*per cent*	*per cent*
0.05	57.0	32.4
0.08	95.0	10.0
0.17	99.6	0.6
0.25	100.0	0.0
0.33	100.0	0.05
0.42	100.0	0.1
0.50	100.0	0.0
0.67	100.0	0.0
0.83	100.0	0.0
1.00	100.0	1.5
1.50	100.0	0.0
2.00	100.0	0.0
3.00	100.0	0.0
4.00	100.0	0.0
5.00	99.0	0.0

[2] Eighty per cent sea water, for example, is made up of eight parts sea water and two parts distilled water. The sea water used was always taken at the same height of tide.

The percentages of cleavage and of activation were based on counts of at least three hundred eggs; frequently, especially for very low or very high percentages of activation, a much larger number were counted.

It was shown that two types of activated eggs appear as a result of the treatment. One type is characterized by initial changes which are indistinguishable from those induced by the sperm. In this type the breakdown of the germinal vesicle, the rounding out of the indentation, the elevation of the membrane, and the extrusion of polar bodies occur in very much the same manner as when the egg is fertilized by a sperm. The time relations for these various changes, allowing for the time of exposure, compare very closely with the time schedule of the same events in the fertilized egg. However, in spite of the remarkable similarity in behaviour of this type of artificially activated egg to that of the fertilized egg, none of the eggs divide.[3]

The other type of artificially activated egg departs widely in its behaviour from that of the normal fertilized egg. The only visible

TABLE II

Unfertilized Eggs Treated with 20 Per Cent Sea Water, Temperature 22.0° C.

Length of Exposure	Activation	Cleavage of Activated Eggs
min.	*per cent*	*per cent*
0.08	25.0	69.2
0.17	58.1	58.7
0.25	92.0	31.4
0.33	98.8	17.3
0.50	100.0	3.1
0.67	100.0	1.0
0.83	100.0	4.2
1.00	100.0	0.5
1.17	100.0	0.1
1.33	99.5	2.2
1.50	100.0	0.1
1.67	100.0	0.0
1.83	100.0	0.0
2.00	100.0	0.0
2.50	100.0	0.0
3.00	100.0	0.0
3.50	100.0	0.0
4.00	100.0	0.0
5.00	100.0	0.0
7.00	100.0	0.1
10.00	100.0	0.3
15.00	100.0	0.6
20.00	100.0	1.5
40.00	100.0	0.0

[3] In only three cases were eggs with two polar bodies seen to divide. The three eggs proceeded only as far as the two-cell stage.

change that occurs in this type of egg within the first three-quarters of an hour after treatment is the dissolution of the germinal vesicle. The egg remains indented, no membrane elevation occurs, and no polar bodies are extruded. After that time the eggs begin to round up, and lift off membranes, but no polar bodies appear. Practically all of the eggs of this type divide, the time of first division varying from one hour and twenty minutes to about three hours. The eggs which cleave and develop are thus the ones which show a poor initial response to the treatment. In what follows, then, the percentage of cleavage is practically identical with the percentage of "poorly activated eggs," and the data on percentage of cleavage and of activation will also show the relation between the percentage of "imperfectly" and of "perfectly" activated eggs for various strengths of hypotonic solutions.

The Variation of Percentage of Activation and of Cleavage with Length of Exposure for Various Dilutions of Sea Water

Treatment with Distilled Water

The action of distilled water is extremely rapid in causing activation of the eggs. After 3 seconds' treatment, 57 per cent of the eggs

Table III

Unfertilized Eggs Treated with 30 Per Cent Sea Water, Temperature 22.1° C.

Length of Exposure	Activation	Cleavage of Activated Eggs
min.	*per cent*	*per cent*
0.17	3.8	37.5
0.33	59.8	13.8
0.50	99.7	1.4
0.67	99.6	0.9
0.83	100.0	0.4
1.00	100.0	0.6
1.33	100.0	0.4
1.67	98.1	3.1
2.00	99.2	0.7
2.50	99.0	0.5
3.00	99.3	0.8
3.50	98.5	1.6
4.00	83.6	2.3
4.50	86.1	3.2
5.00	81.8	11.1
6.00	86.7	1.0
8.00	100.0	0.0
10.00	100.0	0.1
15.00	100.0	0.0
20.00	100.0	0.0
40.00	100.0	0.0

become activated, and after 15 seconds all of the eggs are activated. The results of one series of exposures are given in Table I. Another series run in the same manner gave quite similar results. The percentage of the activated eggs that cleave (column three in the table) is seen to drop very rapidly as the percentage of activation increases. Thus, when 100 per cent activation is obtained, there is practically no cleavage.

Cytolysis sets in after 2 minutes' exposure and reaches 90 per cent at 5 minutes' treatment. The activated eggs in that range are somewhat abnormal in appearance, having a relatively wide membrane and forming blisters over the surface so that polar bodies are often indistinguishable.

Treatment with Twenty Per Cent Sea Water

The action of 20 per cent sea water is less rapid than that of distilled water. The results of one series are given in Table II.

TABLE IV

Unfertilized Eggs Treated with 40 Per Cent Sea Water, Temperature 22.0° C.

Length of Exposure	Activation	Cleavage of Activated Eggs
min.	*per cent*	*per cent*
0.17	0.0	0.0
0.33	52.1	31.4
0.50	94.0	13.5
0.67	97.8	2.3
0.83	99.7	0.9
1.00	99.9	0.1
1.33	100.0	0.1
1.67	100.0	0.2
2.00	99.6	0.0
2.50	99.0	2.4
3.00	98.8	1.1
3.50	98.3	1.6
4.00	97.5	3.2
5.00	85.3	6.4
7.00	88.0	0.9
10.00	99.9	0.4
15.00	100.0	0.0
20.00	100.0	0.0
40.00	100.0	0.0

Fifteen seconds longer treatment is required to give 100 per cent activation than for the distilled water. The rise in percentage of activation with time of exposure is again seen to be accompanied by a drop in cleavage. No exceptions are seen in the first part of the table and the ones occurring in the latter part are of small magnitude. Cytolysis sets in after 4 minutes' exposure and reaches 70 per cent

after 15 minutes and 90 per cent after 20 minutes. Increasing numbers of abnormal eggs of the type described above are found in that range. Two other series of experiments were run, and closely similar results obtained.

TABLE V

Unfertilized Eggs Treated with 45 Per Cent Sea Water, Temperature 21.0° C.

Length of Exposure	Activation	Cleavage of Activated Eggs	Volume in $\mu^3 \times 10^{-6}$
min.	*per cent*	*per cent*	
0.17	0.2	0.0	
0.33	11.5	50.0	
0.50	39.0	29.2	
0.67	81.3	10.6	
0.83	92.9	4.8	
1.00	98.6	1.4	8.12
1.33	100.0	0.2	
1.67	100.0	0.0	
2.00	99.5	0.0	8.90
2.50	98.3	0.3	
3.00	96.4	0.8	9.51
3.50	87.3	2.2	
4.00	65.7	4.0	10.10
5.00	34.4	20.5	10.45
6.00	21.2	26.2	10.92
8.00	7.9	22.2	11.49
10.00	6.9	50.0	11.92
15.00	0.8	43.0	12.95
20.00	1.3	51.6	13.51
40.00	3.3	8.3	13.96

Treatment with Thirty Per Cent Sea Water

With 30 per cent sea water the percentage of activation rises less rapidly than with 20 per cent. The results again show that as the percentage of activation increases, the percentage of cleavage decreases. Table III gives the results of one series. The percentage of activation shows a slight drop after about one and one-half minutes' exposure which becomes quite marked at 4 to 6 minutes' exposure. But as the activation drops, the cleavage is seen to rise, so that at 5 minutes' exposure, where the activation has dropped to 82 per cent, the cleavage has risen to 11 per cent. The activation then rises again to 100 per cent and the cleavage drops to zero.

Cytolysis sets in after 6 minutes' treatment and reaches 50 per cent after 40 minutes. The abnormal eggs referred to above again appear in this range of exposures.

Four other series of experiments were run, at temperatures ranging from sixteen to twenty-three degrees, and very similar results obtained.

The inverse relation between cleavage and activation was evident in each series. If every case in which an increase (or decrease) in activation accompanied by an increase (or decrease) in cleavage to the extent of at least one per cent is considered an exception, then out of a total of eighty-one dishes there are seven exceptions.

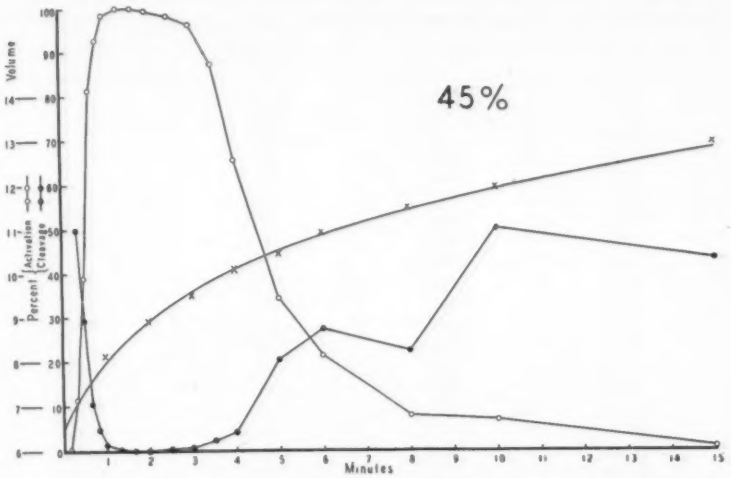

FIG. 1. Variation of percentage activation (open circles), percentage cleavage (solid circles) and mean volume of eggs (continuous curve) with length of exposure to 45 per cent sea water. Data of Table V.

Treatment with Forty Per Cent Sea Water

The percentage of activation for eggs treated with 40 per cent sea water rises less rapidly than for eggs treated with any of the preceding dilutions. Table IV gives the results of one series of exposures. The activation is seen to rise rapidly to 100 per cent, drop more slowly to 85 per cent and return again to 100 per cent. The percentage of cleavage of the activated eggs decreases as the activation increases, and increases as the activation drops. The inverse relation between cleavage and activation is thus again clearly shown, only one exception occurring in the table, namely at the three minute exposure, where a drop in activation is followed by a drop in cleavage greater than one per cent.

At the 8 minutes' exposure there is 4 per cent of cytolysis, which increases to about 30 per cent for the 40 minutes' treatment. The abnormal eggs again occur in this range.

Three other series were run with 40 per cent sea water, totaling forty-seven dishes. Out of these a total of four exceptions of magnitude greater than one per cent were obtained.

TABLE VI

Unfertilized Eggs Treated with 50 Per Cent Sea Water, Temperature 21.5° C.

Length of Exposure	Activation	Cleavage of Activated Eggs	Volume in $\mu^3 \times 10^{-6}$
min.	*per cent*	*per cent*	
0.17	0.1	100.0	
0.33	2.0	80.1	
0.50	43.2	40.6	
0.67	91.0	21.6	
0.83	96.7	10.6	
1.00	99.2	5.3	7.83
1.33	99.8	0.7	
1.67	100.0	0.4	
2.00	100.0	0.4	8.72
2.50	100.0	1.2	
3.00	90.8	26.9	9.29
3.50	93.4	21.9	
4.00	86.0	39.2	9.72
4.50	68.3	63.2	
5.00	33.9	76.2	10.10
6.00	34.9	80.0	10.46
8.00	18.8	94.7	11.08
10.00	7.1	83.3	11.42
15.00	0.5	40.0	12.20
20.00	0.4	79.0	12.72
40.00	1.5	61.3	12.85

Treatment with Forty-five Per Cent Sea Water

The results obtained with 45 per cent sea water differ in two respects from those obtained with the preceding dilutions of sea water. These are, first, that the percentage of activation returns practically to zero after its initial rise to 100 per cent, and second, that very little cytolysis sets in.

In Table V the results of one series of experiments are presented. The rate of increase in activation is slower than with the preceding dilutions. After 8 minutes' exposure a few of the eggs become cytolysed and the amount of cytolysis reaches 5 per cent after 40 minutes.

The inverse relation between cleavage and activation is quite evident in the table and is illustrated graphically in Fig. 1. The "exceptions" generally occur in the dishes showing low percentages of activation. Similar results were obtained in three other series of experiments run with 45 per cent sea water. Out of a total of seventy-

three dishes examined, sixteen exceptions were found, all of them in dishes showing less than 8 per cent activation.

The increase in activation occurs much more rapidly than the decrease. This may readily be seen in the graph (Fig. 1), where the percentage of activation plotted against time gives a skew curve. The probable interpretation of this result will be presented later. But in connection with the activation-time curve, it is of interest to

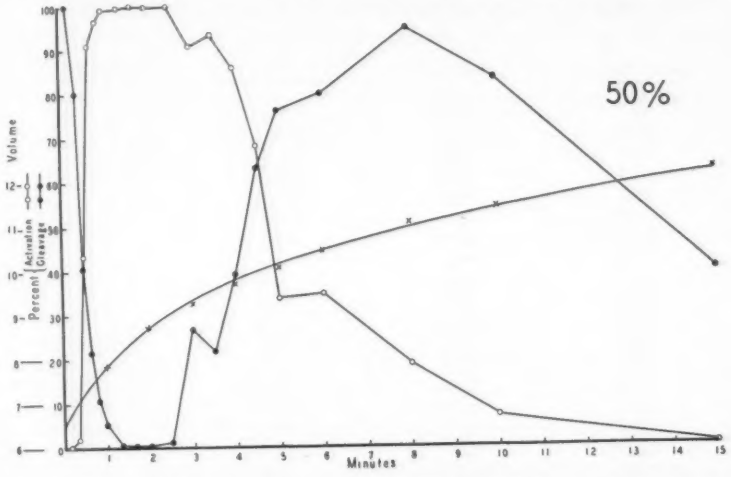

FIG. 2. Variation of percentage activation (open circles), percentage cleavage (solid circles) and mean volume of eggs (continuous curve) with length of exposure to 50 per cent sea water. Data of Table VI.

present here the curve showing the increase in volume with length of exposure to the 45 per cent sea water. The data from which the curve was drawn are given in Table V. Each point represents the average of the volumes of three eggs. The measurements of the diameters were made with a Filar ocular micrometer. With this micrometer measurements accurate to 0.1 per cent may be obtained. However, the variations in volume, for the data presented here and below, ranged as high as 5 per cent. This is probably due to the rapid change in volume that is taking place as the measurements are made and to the variability of the eggs. The volume measurements are being repeated on a larger scale and by means of a cinematograph in order to obtain accurate data for an analysis of the swelling process itself. But even the relatively rough data presented here will be shown to be useful in an analysis of the activation-time curves obtained in these experiments.

The swelling curve of Fig. 1 shows that the eggs continue to increase in volume even after the percentage of activation begins to drop. The curve itself is of the exponential type, the slope continually decreasing. In other words, the increase in volume occurs less rapidly as the time of exposure increases.

TABLE VII

Unfertilized Eggs Treated with 55 Per Cent Sea Water, Temperature 21.2° C.

Length of Exposure	Activation	Cleavage of Activated Eggs	Volume in $\mu^3 \times 10^{-6}$
min.	*per cent*	*per cent*	
0.17	0.4	100.0	
0.33	0.9	100.0	
0.50	10.0	53.3	
0.67	38.4	37.4	
0.83	70.3	29.4	
1.00	89.5	17.6	7.74
1.33	96.2	9.6	
1.67	98.4	2.0	
2.00	99.9	1.0	8.73
2.50	97.8	1.6	
3.00	93.3	5.6	9.32
3.50	88.8	7.1	
4.00	85.6	12.4	9.57
5.00	66.4	21.3	9.82
6.00	64.1	21.0	10.11
8.00	47.3	16.6	10.49
10.00	13.9	15.4	10.89
12.00	1.7	44.2	
15.00	1.1	70.6	11.41
20.00	0.0	0.0	11.63
40.00	2.2	90.9	11.84

Treatment with Fifty Per Cent Sea Water

The results obtained with 50 per cent sea water are quite similar to those obtained with 45 per cent sea water, except that the increase in activation occurs more slowly and practically no cytolysis occurs in any of the dishes. The increase in volume of the eggs in 50 per cent sea water is also somewhat slower than for those exposed to 45 per cent sea water, and the equilibrium volume attained is, of course, smaller.

Table VI contains the results of one series of experiments and a set of volume measurements (means of three eggs) obtained at different times. The data are presented graphically in Fig. 2. The results again bear out the inverse relation between cleavage and activation. It is interesting to note that the irregular rise in the activation curve

at the three and one-half minutes' exposure is accompanied by a drop in cleavage. The exceptions occur only in the last four points of the graph, where the percentage of activation is low. A total of eighty-two dishes in five series of experiments gave sixteen exceptions all of the same type illustrated here.

The activation shows a drop to practically zero after its initial rise

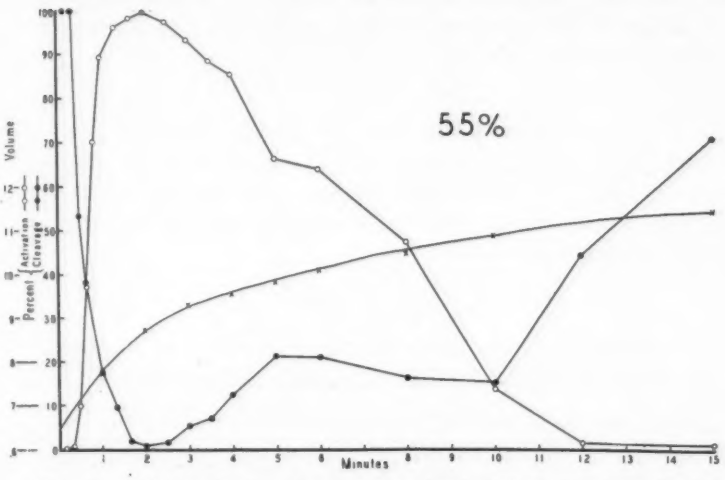

FIG. 3. Variation of percentage activation (open circles), percentage cleavage (solid circles) and mean volume of eggs (continuous curve) with length of exposure to 55 per cent sea water. Data of Table VII.

to 100 per cent, as in the preceding case. The rise in activation is again seen to occur more rapidly than the subsequent decrease, giving the skew activation-time curve shown in Fig. 2. The volume curve for the eggs in the 50 per cent sea water is of the same type as obtained with the preceding dilution.

Treatment with Fifty-five Per Cent Sea Water

The results of one series of experiments with 55 per cent sea water and the volume data for the same dilution are presented in Table VII and Fig. 3. The data again show a decrease in cleavage as the activation increases and an increase in cleavage as the activation decreases. Four series of experiments totaling seventy-four dishes gave twelve exceptions—chiefly at low percentages of activation.

The activation-time curve is of the same shape as that obtained in the preceding case, but it is shifted slightly to the right, so that the time required for the maximum percentage of activation and for the return to zero per cent activation is longer than with 50 per cent sea water.

The swelling curve (Fig. 3) shows that the volume continues to increase after the percentage of activation has reached a maximum. It is also of the exponential type in which the rate of increase in volume decreases with time.

Treatment with Sixty Per Cent Sea Water

The results obtained with 60 per cent sea water again differ from the preceding only in the time relations of activation and cleavage and the volume curve. The data is given in Table VIII, and graphically represented in Fig. 4.

TABLE VIII

Unfertilized Eggs Treated with 60 Per Cent Sea Water, Temperature 22.3° C.

Length of Exposure	Activation	Cleavage of Activated Eggs	Volume in $\mu^3 \times 10^{-6}$
min.	*per cent*	*per cent*	
0.17	0.1	0.0	
0.33	1.0	0.0	
0.50	1.0	50.0	
0.67	13.4	33.3	
0.83	30.3	25.8	
1.00	61.3	25.9	7.88
1.33	73.1	13.7	
1.67	88.7	7.7	
2.00	99.4	0.4	8.68
2.50	100.0	0.1	
3.00	100.0	0.2	9.16
3.50	97.6	2.9	
4.00	88.2	9.3	9.45
5.00	85.7	5.5	9.69
6.00	75.0	30.6	10.01
7.00	42.4	46.5	
8.00	16.0	61.5	10.16
10.00	14.7	81.2	10.49
12.00	9.5	37.1	
15.00	5.1	72.3	10.94
20.00	0.0	0.0	11.10
40.00	0.0	0.0	11.11

The cleavage-activation relation shows up quite clearly. Out of eighty-nine dishes in five series of experiments, fourteen relatively unimportant exceptions were obtained.

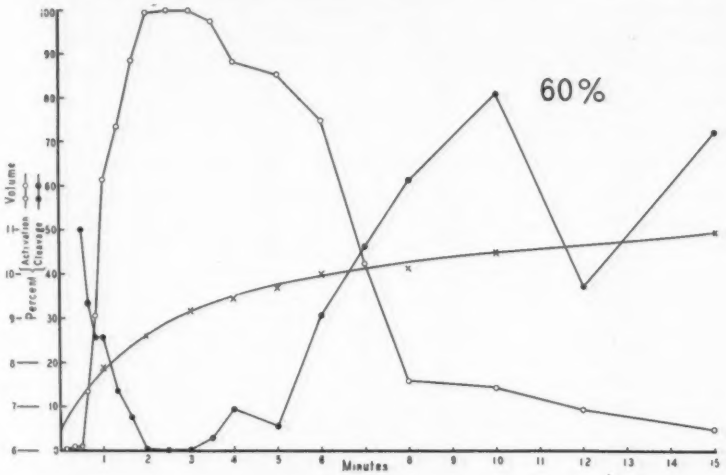

FIG. 4. Variation of percentage activation (open circles), percentage cleavage (solid circles) and mean volume of eggs (continuous curve) with length of exposure to 60 per cent sea water. Data of Table VIII.

The activation-time curve shows a slight shift to the right when compared with the preceding ones, but its asymmetry is still quite evident.

The swelling curve is of the same type as in the preceding cases but approaches a lower equilibrium volume.

Treatment with Sixty-five Per Cent Sea Water

Sixty-five per cent sea water gives results which differ from the preceding in the same direction as the results obtained with the 60 per cent sea water differ from those obtained with 55 per cent. One series of experiments and a set of mean volumes are shown in Table IX and Fig. 5.

The inverse relation between cleavage and activation is evident in spite of certain relatively large irregularities. From three series of experiments seven relatively large and eight minor exceptions were obtained out of a total of fifty-nine dishes.

TABLE IX

Unfertilized Eggs Treated with 65 Per Cent Sea Water, Temperature 22.0° C.

Length of Exposure	Activation	Cleavage of Activated Eggs	Volume in $\mu^3 \times 10^{-5}$
min.	*per cent*	*per cent*	
0.17	0.0	0.0	
0.33	0.2	0.0	
0.50	0.0	0.0	
0.67	0.7	40.0	
0.83	6.4	36.3	
1.00	13.6	11.8	7.61
1.33	77.8	20.8	
1.67	87.6	13.4	
2.00	92.0	10.2	8.32
2.50	96.5	6.1	
3.00	98.6	2.1	8.73
3.50	92.5	6.7	
4.00	71.9	22.5	9.25
5.00	49.0	56.7	9.56
6.00	21.8	46.9	9.49
7.00	20.3	51.0	
8.00	13.8	42.4	9.88
10.00	13.7	34.9	10.15
12.00	6.0	40.9	
15.00	3.9	15.3	10.50
20.00	0.2	100.0	10.55
40.00	0.0	0.0	10.55

The activation-time curve (Fig. 5) is asymmetrical as in the preceding cases, but it shows a slight shift to the right.

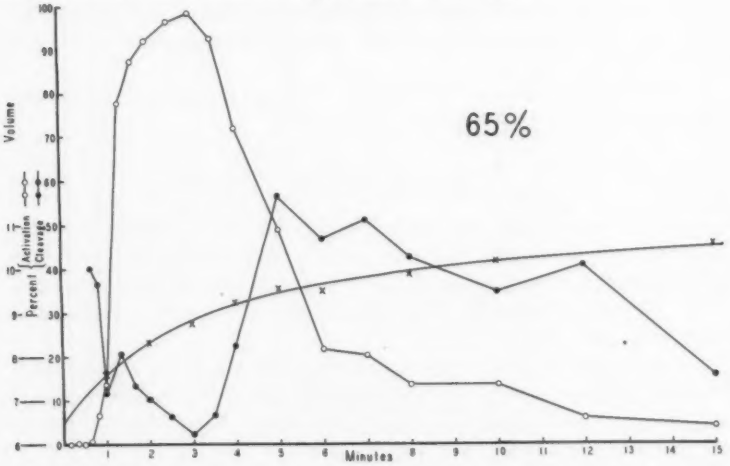

FIG. 5. Variation of percentage activation (open circles), percentage cleavage (solid circles) and mean volume of eggs (continuous curve) with length of exposure to 65 per cent sea water. Data of Table IX.

The volume increase is slower than for the eggs in more dilute sea water, and approaches a lower asymptotic value.

Treatment with Seventy Per Cent Sea Water

The results of one series run with 70 per cent sea water are tabulated in Table X. Figure 6 shows that trend of the data graphically.

TABLE X

Unfertilized Eggs Treated with 70 Per Cent Sea Water, Temperature 21.2° C.

Length of Exposure	Activation	Cleavage of Activated Eggs
min.	*per cent*	*per cent*
0.17	0.4	57.1
0.33	0.8	50.0
0.50	2.5	55.2
0.67	2.4	29.8
0.83	4.1	30.2
1.00	4.6	29.5
1.33	6.4	20.3
1.67	20.4	19.5
2.00	46.1	10.2
2.50	96.2	9.1
3.00	100.0	2.3
3.50	97.5	10.9
4.00	69.2	19.7
4.50	51.6	35.7
5.00	31.6	55.5
6.00	16.7	65.2
8.00	7.4	82.9
10.00	13.3	76.3
15.00	12.7	85.8
20.00	4.6	83.3
40.00	0.3	66.7

No serious divergence from the cleavage-activation relation is evident. Two series of experiments totaling thirty-seven dishes gave seven minor variations.

The activation-time curve (Fig. 6) is again decidedly asymmetrical. It is displaced to the right, so that the return to zero per cent activation requires a longer exposure than in the preceding cases.

The volume data are not presented for this or for the succeeding dilutions of sea water. The volume increase proceeds more slowly, of course, and reaches a smaller equilibrium volume with increasing concentrations of sea water.

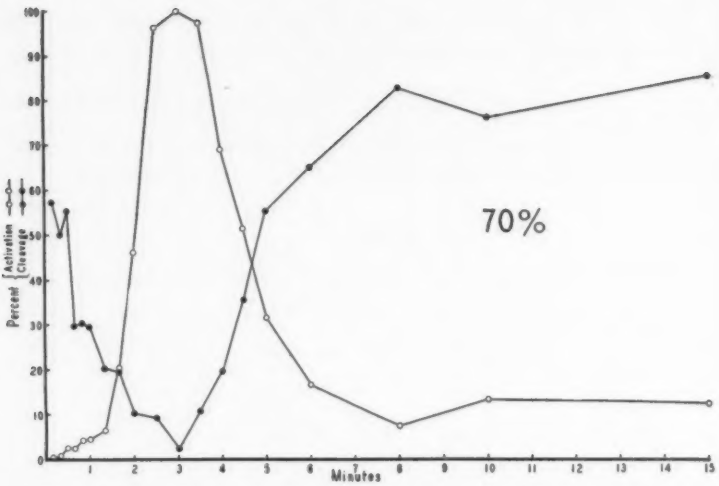

FIG. 6. Variation of percentage activation (open circles) and percentage cleavage (solid circles) with time of exposure to 70 per cent sea water. Data of Table X.

Treatment with Seventy-five Per Cent Sea Water

Table XI and Fig. 7 contain the results of one series of experiments with 75 per cent sea water.

TABLE XI

Unfertilized Eggs Treated with 75 Per Cent Sea Water, Temperature 20.8° C.

Length of Exposure	Activation	Cleavage of Activated Eggs
min.	*per cent*	*per cent*
0.17	0.0	0.0
0.33	0.0	0.0
0.50	0.0	0.0
0.67	0.0	0.0
0.83	0.0	0.0
1.00	0.0	0.0
1.33	3.3	71.4
1.67	8.2	50.0
2.00	33.9	45.9
2.50	83.2	49.5
3.00	86.2	32.3
3.50	90.0	28.4
4.00	79.3	28.6
5.00	47.8	59.2
6.00	30.2	65.0
8.00	7.4	61.3
10.00	1.2	66.7
15.00	0.1	40.0
20.00	0.0	0.0

As before, the percentage of cleavage varies inversely with the percentage of activation, although the difference between the maximum of activation and the corresponding minimum of cleavage is not as great as in the cases listed above. Two series of experiments totaling thirty-one dishes gave four exceptions.

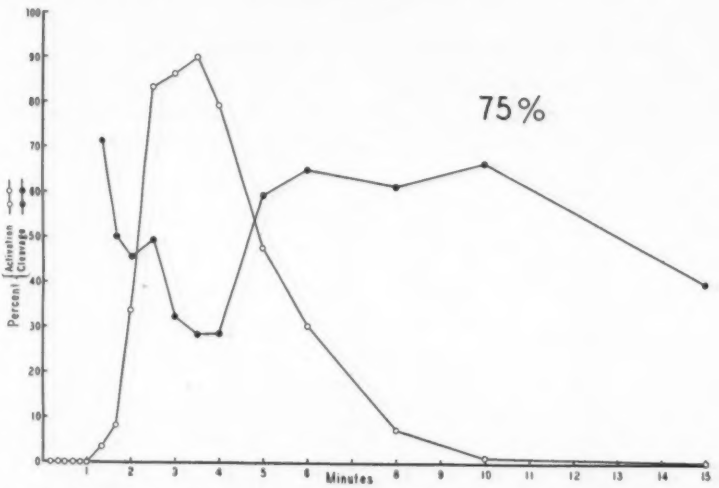

FIG. 7. Variation of percentage activation (open circles) and percentage cleavage (solid circles) with time of exposure to 75 per cent sea water. Data of Table XI.

For the activation-time curve (Fig. 7), the time to reach a maximum is longer than in the preceding case, but the drop to zero per cent occurs sooner. However, the maximum value reached is only 90 per cent activation as compared with 100 per cent in the previous cases. The curve itself is still asymmetrical.

Treatment with Eighty Per Cent Sea Water

Eighty per cent sea water generally fails to give more than one to two per cent activation. In one series of experiments, however, an exceptionally high percentage of activation was obtained. The results are given in Table XII and Fig. 8.

It is readily seen from the data that practically every increase (or decrease) in activation is accompanied by a decrease (or increase) in cleavage, bearing out the inverse relation between cleavage and activation.

The activation-time curve reaches its maximum as quickly as for the 75 per cent sea water, but that is undoubtedly due to the higher temperature at which this series was run. The activation curve does not return to zero, but maintains a relatively high percentage of activation and a correspondingly high percentage of cleavage.

TABLE XII

Unfertilized Eggs Treated with 80 Per Cent Sea Water, Temperature 22.5° F.

Length of Exposure	Activation	Cleavage of Activated Eggs
min.	*per cent*	*per cent*
0.17	0.0	0.0
0.33	0.0	0.0
0.50	0.0	0.0
0.67	0.0	0.0
0.83	0.2	100.0
1.00	1.4	42.8
1.33	6.6	31.2
1.67	14.5	30.8
2.00	58.2	26.8
2.50	94.1	21.5
3.00	95.7	17.6
3.50	98.8	3.5
4.00	98.7	8.3
5.00	81.6	57.8
6.00	92.5	37.1
8.00	80.7	48.6
10.00	65.5	59.1
15.00	64.1	38.2
20.00	62.5	48.8
40.00	47.6	64.2

THE VARIATION OF PERCENTAGE OF ACTIVATION WITH VOLUME FOR VARIOUS DILUTIONS OF SEA WATER

When the percentage of activation is plotted against the mean volume attained by the eggs at different lengths of exposure, a curve is obtained which is much more symmetrical than the activation-time curve. Figure 9 shows five curves of that type, for 45, 50, 55, 60 and 65 per cent sea water. The percentages of activation were plotted in each case against the volumes attained at corresponding times of exposures, the volumes being taken from the smooth curves.

The activation-volume curves of Fig. 9 approach in shape the normal distribution curve. The individual curves have the same abscissa but the ordinates are raised successively for each dilution of sea water. It can readily be seen that even with the same coördinates

the curves would not coincide; but their divergence is no greater than would be expected when one considers the statistical nature of the activation values and the errors involved in the volume measurements. Moreover, there are probably injury factors operative in the lower concentrations of sea water that are not present in the higher concentrations, as indicated by the cytolysis obtained in 45 per cent sea water.

Fertilization of "Over-Exposed" Eggs

The activation-time curves for concentrations of sea water above 40 per cent are seen to rise to a maximum of about 100 per cent activation and then drop off to zero.

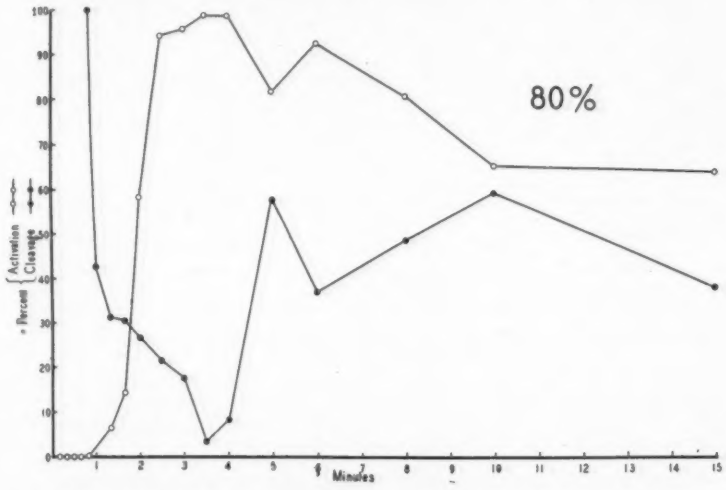

Fig. 8. Variation of percentage activation (open circles) and percentage cleavage (solid circles) with time of exposure to 70 per cent sea water. Data of Table XII.

The eggs which do not respond before the "optimum exposure" is reached may be termed "under-exposed" unactivated eggs, and those which do not respond upon longer exposures may be termed "over-exposed" unactivated eggs.

The failure of the "over-exposed" unactivated eggs to respond to the treatment might presumably be due to an injury effect, or other change produced in the eggs. The "over-exposed" unactivated eggs as well as the under-exposed unactivated eggs were therefore inseminated with fresh sperm in order to determine whether they would become fertilized and produce normal embryos. The results obtained

with 45 to 65 per cent sea water are given in Table XIII. The third column in the table gives the total percentages of activation obtained with the lengths of exposure listed in column two. The fourth column gives the percentage of the unactivated eggs that become fertilized upon addition of sperm, and the fifth column, the percentage of the fertilized eggs that produce normal larvæ.

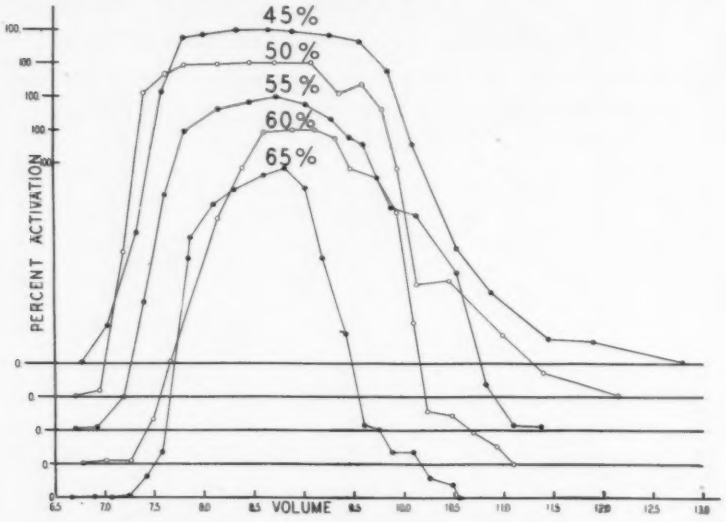

FIG. 9. Variation of percentage of activation with mean volume of eggs attained at corresponding times of exposure to 65, 60, 55, 50, and 45 per cent sea water. Ordinates raised successively for each dilution of sea water. Data from Tables V to IX; volumes taken from the smooth curves of Figs. 1 to 5.

The unactivated eggs were transferred to a separate dish and inseminated at about 2 to 3 hours after treatment. Control eggs (listed in the table as 0.0 minutes' exposure) were inseminated at the same time.

The "under-exposed" unactivated eggs are not given for the 55 and the 60 per cent sea water. In the other three cases the "under-exposed" unactivated eggs show practically 100 per cent fertilization and a high percentage of normal embryos. The "over-exposed" eggs show a high percentage of fertilization in every case, comparing quite favorably with that given by the control eggs. The percentage of normal embryos obtained varies considerably, but is quite as good as that obtained from the controls, except for the 45 per cent sea water. However, in the latter case a relatively large percentage of the eggs were polyspermic.

TABLE XIII

Insemination of "Under-Exposed" and "Over-Exposed" Unactivated Eggs

Concentration of Sea Water	Length of Exposure	Activation	Fertilization	Normal Embryos
per cent	*min.*	*per cent*	*per cent*	*per cent*
	0.0	—	95	40
	0.17	0.0	99	50
	1.50	100.0	—	—
45	10.00	5.7	70	20
	15.00	0.0	60	2
	20.00	0.3	60	5
	40.00	0.5	60	5
	0.0	—	98	25
50	0.17	0.1	100	60
	2.00	100.0	—	—
	15.00	0.5	80	70
	0.0	—	65	70
55	2.00	100.0	—	—
	20.00	0.0	50	65
	0.0	—	100	100
	2.75	100.0	—	—
60	7.00	42.4	100	100
	10.00	14.7	100	95
	20.00	0.0	100	100
	0.0	—	100	65
	0.17	0.0	100	100
65	3.00	99.0	—	—
	7.00	28.1	100	40
	10.00	18.3	90	75
	20.00	0.0	99	50

The results show that the "over-exposed" unactivated eggs are still capable of becoming fertilized, even though a shorter exposure would have resulted in every egg becoming activated upon return to normal sea water.

DISCUSSION

1. *Variation of Rate of Increase in Activation with Dilution of Sea-Water*

It is evident from the results presented above that the factors causing activation are brought into action more quickly, the lower the concentration of the sea water used for the treatment. In the dilute sea water the egg swells due to intake of water. The volume increase also occurs more quickly, the lower the concentration of the sea water in which the eggs are allowed to swell. This parallel behaviour suggests that volume increase in the dilute sea water may be

used as a basis for an interpretation of the results presented above; but this is not meant to imply that water-intake alone is responsible for the activation of the egg.[4]

2. *Activation-Time Curves*

For concentrations of sea water ranging from 45 to 75 per cent the percentage of activation was seen to rise rapidly to a maximum and then fall off more slowly. In terms of volume change this means that when the egg is in a definite volume range it will become activated upon return to normal sea water, but before or after passing through that volume range the egg does not become activated upon return to normal sea water. This volume range is evidently well below the equilibrium volume, since the eggs continue to swell after the time of exposure giving the maximum activation. The reason why a range of volumes rather than one definite volume is specified will be indicated below. There is considerable variability in the time at which different eggs pass through the same volume range when a given batch is exposed to a given dilution of sea water. Thus some of the eggs will have reached the volume range from which return to normal sea water results in activation before the others have entered that range. Correspondingly, some of the eggs will have passed through that volume range while the others are still in it. Let us term the volume range resulting in activation the "optimum volume range." The percentage of eggs passing through a given volume range at a given time will depend on the kind of variability shown by the eggs. If the variability of this material is expressed by the normal distribution curve, then we would expect the variability in the percentage of eggs passing through the "optimum volume range" to be expressed by that type of curve only if the increase in volume were a linear function of the time of exposure. But the volume increase is a logarithmic function of time, the rate of swelling continually decreasing with time of exposure. The eggs therefore enter the "optimum volume range" more rapidly than they leave it. Thus the variation in the percentage of eggs passing through the "optimum volume range" with time of exposure should be expressed by a skew distribution curve with its mode displaced to the left. In other words, the variation of percentage activation with time of exposure should give a skew curve, since the percentage of eggs passing through the "optimum volume range" is by definition identical with the percentage of activation. This is in fact the type of curve that is

[4] The change in hydrogen ion concentration, for example, might be an important factor. It ranged from pH 8.2 for the sea water to pH 7.1 for the distilled water used.

obtained when percentage of activation is plotted against length of exposure (Figs. 1 to 8).

The reason for assuming a range of volumes rather than one definite optimum volume results from the following consideration. The maximum of the activation-time curve is at 100 per cent activation. This means that all of the eggs must be in such a condition after a certain time of exposure that removal to normal sea water at that time results in every egg becoming activated. But the volume measurements show that the eggs vary in the time of exposure at which a given volume is reached. Therefore, if we adhere to the volume interpretation we must assume that a range of volumes, at least as great as the variation in volume of the individual eggs, is effective in causing activation upon return to ordinary sea water. The time of exposure at which all of the eggs are in that "optimum volume range" then results in 100 per cent activation.

On this basis the more rapid swelling obtained with progressively lower concentrations of sea water should cause a shifting of the activation-time curve to the left proportional to the increase in rate of swelling and likewise a shortening of the time range of activation. The results presented above show that this is in general true. But with extreme dilutions of sea water (40 per cent to distilled water) the drop to zero per cent activation does not occur. This is probably due to a secondary effect as indicated by the fact that there is a tendency for the activation to drop (see Tables II, III, IV), but as cytolysis sets in a second rise in activation (of an abnormal type) takes place.

One should also expect, according to the volume interpretation, that the concentration of sea water in which the equilibrium volume of the eggs is within the "optimum volume range" should give an activation-time curve that does not drop. This is presumably approached by the 80 per cent sea water (Table XII and Fig. 8).

3. *Activation-Volume Curves*

If the variation of the percentage of activation with time of exposure is correlated with the variation in volume of the eggs attained at corresponding times of exposure, then the percentage of activation plotted against mean volume should give a normal distribution curve, which should be identical for the various dilutions of sea water. The results show that this is roughly true. The curves obtained with various dilutions of sea water (Fig. 9) are quite symmetrical when compared with the activation-time curves. The probable reasons for the failure of the various curves to be exactly identical have been given above.

The expectation of a normal distribution curve for percentage activation plotted against mean volume is based on the assumption that the variation in volume of the eggs, at each time of exposure considered, is expressed by the normal probability curve. This is the type of variation that is generally assumed for biological material in the absence of further information. To obtain such information in this case it would be necessary to measure the volumes of a large number of eggs at various times of exposure. This has not been done on a large enough scale and accurately enough to determine whether the chance law holds for the volumes at every exposure used, but the measurements obtained on untreated eggs indicate that their variation in volume is of that type.

4. *"Over-Exposed" Unactivated Eggs*

It has been shown that the over-exposed unactivated eggs obtained with solutions ranging from 45 per cent to 65 per cent sea water can still be fertilized and may produce normal embryos. This may be taken to mean that the eggs have not been irreversibly affected by treatment with these dilutions of sea water. Consonant with this fact is the observation previously reported, that no visible changes aside from the swelling are seen to occur in the treated eggs while in the dilute sea water. It is also in accord with the result that the time for the initial stages (*e.g.* polar body extrusion) of the artificially activated eggs is comparable with that of the fertilized eggs only if allowance is made for the time of treatment.

It is evident then that no developmental changes occur in the egg while in the hypotonic solution, but that activation is initiated by the return to normal sea water after a definite time of exposure (or after a certain amount of water has been taken in). The question may therefore be raised as to why a longer exposure fails to evoke a response in the egg upon return to normal sea water when a shorter one does. If the egg were found to be injured by the longer exposure this question might be more readily answered. But the data presented here show that this is not so. The question bears directly on the mechanics of activation. With the data available we can only answer by restating the result in the following terms—that a definite change (enabling the egg to become activated upon return to normal sea water) *is* produced in the egg by the intake of an amount of water within a certain range, but that the change is reversed when more water is taken in. In other words, by the difference in behaviour upon return to normal sea water, an egg in the optimum exposure range must be intrinsically different from an egg in the earlier or later ranges, and by the similarity

in behaviour upon return to normal sea water, an egg in the earlier range of exposures must be intrinsically the same (neglecting the manifest difference in volume) as an egg in the later range; hence the change produced must be reversed.

The return to original condition of eggs that have been allowed to swell in dilute sea water has also been noted in eggs of *Nereis* (Just, 1930) and eggs of *Arbacia* (McCutcheon and Lucké, 1926). But in neither of these cases is it stated whether activation is obtained at shorter exposures.

The ability of eggs that have been "over-exposed" to butyric acid to become fertilized has been noted by Moore (1916) for *Arbacia*, Just (1919) for *Echinarachnius*, and Lillie (1921) for *Strongylocentrotus*. But in these cases the cleavage and development were stated to be abnormal.

5. *Cleavage-Activation Relation*

The inverse relation between the percentage of cleavage and the total percentage of activation may now be interpreted in a similar way provided we introduce a "sub-optimum volume range" on both sides of the "optimum volume range." The justification of this arises from a consideration of the results reported in a previous publication (Tyler, 1931). It was shown that the activated eggs that extrude both polar bodies practically never divide, even though the response of that type of egg to the treatment is outwardly indistinguishable from the response of the egg to the sperm. Only the eggs that produce no polar bodies were the ones to cleave, but such eggs were shown to respond in a relatively very slow and abnormal fashion to the treatment in respect to the breakdown of the germinal vesicle, rounding out of indentation, and membrane elevation. Such is the type of result one would expect from a "sub-optimum" treatment. In terms of volume change this "sub-optimum" exposure would be obtained in a "sub-optimum volume range." Practically no eggs of that type are obtained at the time of exposure giving 100 per cent activation, but they occur in increasing numbers to either side of that exposure time. Since, at the time of exposure giving 100 per cent activation, all of the eggs are assumed to be in the "optimum volume range," the "sub-optimum volume range" must occur on each side of the former.[5]

Thus, when a batch of eggs is treated with dilute sea water, the eggs will pass through a "sub-optimum volume range" both before and after entering the "optimum volume range." At relatively short

[5] The "sub-optimum volume range" must evidently be shorter than the range of variability of the volumes of the eggs, since 100 per cent cleavage (with 100 per cent activation) is never obtained for any given exposure.

times of exposure, then, one would expect most of the activated eggs to be within the "sub-optimum volume range," and so give a high percentage of cleavage (of the activated eggs). But with longer exposures as the total activation increases one would expect more and more of the eggs to enter the "optimum volume range" and so give a low percentage of cleavage. The results would then be reversed upon passing through the second "sub-optimum volume range" with longer exposures.

This leads to a relation between percentage of cleavage and percentage total activation that is identical with that described in the text.

This interpretation can be tested in a much better fashion by following the volume changes of individual eggs in various dilutions of sea water and noting their behaviour when removed to normal sea water after having been allowed to swell to various volumes. Such experiments are now in progress.

The results reported here have an important bearing on what is generally termed the "optimum treatment" in parthenogenesis experiments. It has generally been assumed that the treatment producing the highest percentage of activation (similar to that produced by the sperm), and of cleavage and development is the optimum treatment. But in *Urechis* it has been shown that the treatment that is optimum for activation is not so for cleavage and development. Thus, if one wishes to produce the most parthenogenetic development, the length of exposure used is different from that which would be chosen if one wished to produce the highest percentage of eggs whose initial response to the treatment was most similar to that induced by the sperm. It is preferable, I think, to term the latter the optimum treatment, for the reasons stated above. The failure of eggs receiving the optimum treatment to divide is probably connected with insufficient chromatin (since all such eggs extrude two polar bodies and are left with the haploid number of chromosomes). It should be possible then to produce cleavage in such eggs by suppressing the polar divisions. This is somewhat difficult to accomplish without initiating other changes in the eggs, but the results obtained thus far indicate that suppression of the polar divisions of the "optimally" stimulated eggs results in cleavage.

The inverse relation between percentage of cleavage and percentage of activation appears then to depend on the fact that only the "poorly activated eggs" which extrude no polar bodies are the ones to divide. Thus the extent to which this relation is general for eggs of various forms will probably depend on whether or not the eggs that extrude

both polar bodies divide. In eggs of the sea urchin type, where the polar bodies are extruded in the ovary and where cleavage is apparently possible with the haploid number of chromosomes, we might not expect this relation to hold.

In eggs of *Thalassema neptuni*, which, from the descriptions are very similar to the *Urechis* eggs, artificial activation by means of isotonic solutions has been reported by Hobson (1928). The variations of percentage of activation and of percentage of cleavage [6] are presented for several short series of exposures, but Hobson thinks that the results show an increase in cleavage with increase in activation. However, he notes (pp. 73 and 74) that the maximum of cleavage often fails to coincide with the maximum of activation, when both composition of medium and length of exposure are varied.

Summary

1. The rate of increase in percentage activation of *Urechis* eggs with hypotonic sea water is shown to decrease as the concentration of sea water used is increased from distilled water to 80 per cent sea water.

2. The rate of increase in volume also decreases with increased concentration of sea water.

3. For dilutions of sea water ranging from 75 per cent to 45 per cent, the activation passes through a maximum (usually 100 per cent) and then returns to zero per cent with longer exposures. For lower concentrations of sea water the return to zero per cent is not obtained, but a high percentage of activation is maintained. With 80 per cent sea water the return to zero per cent activation also does not occur.

4. The activation-time curves for 75 per cent to 45 per cent sea water are of the form of skew distribution curves, rising rapidly to 100 per cent activation and falling more slowly to zero per cent.

5. The activation-volume curves are presented for 65 per cent to 45 per cent sea water and are of the form of a normal probability curve. They are roughly identical for the various dilutions of sea water.

6. Practically every series of experiments shows an inverse relation between the percentage of total activation and percentage of cleavage (of the activated eggs); so that as the percentage of activation increases with time of exposure, the percentage of cleavage decreases, and when

[6] Hobson's total activation does not include cleavage. It is not stated in the paper whether the percentage of cleavage is that of all the eggs or of the activated eggs, though it seems to be the former. When the data of his tables is recalculated on this basis, there are thirteen cases in which an increase (or decrease) in activation is accompanied by an increase (or decrease) in cleavage and six cases in which the inverse relation holds.

the percentage of activation decreases with exposure the percentage of cleavage increases.

7. The over-exposed unactivated eggs are still capable of fertilization and of producing normal embryos in spite of the fact that a shorter exposure would have resulted in their becoming activated upon return to normal sea water.

8. The variation in rate of activation with concentration of sea water, the type of activation-time curves, the activation-volume curves, and the fertilization of over-exposed eggs are shown to be interpretable on the basis of volume change occurring in the dilute sea water, a definite volume range being optimum for activation. The cleavage-activation relation is shown to be the outcome of the previously reported result that only the "poorly activated" eggs divide, and its interpretation, based also on the exposures producing such eggs, involves the assumption of a "sub-optimum volume range" on both sides of the optimum.

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THE OCCURRENCE OF MELANOPHORES IN CERTAIN EXPERIMENTAL WOUNDS OF THE GOLDFISH (*CARASSIUS AURATUS*)[1]

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While studying in the goldfish the repair of experimental wounds, crushes, burns, and fractures, it became apparent that melanophores developed in the wounds a few days after the trauma and later degenerated and thus disappeared. Not alone did these melanophores occur directly at the site of the injury, but not infrequently in the corium of adjacent areas and even in remote cutaneous regions. In none of these places were black pigmented cells seen by a previous low-power microscopic examination of the living fishes used for the experiment, nor were melanophores of the corium noticeable by high magnification in sections of tissue removed from the region of the wound at the time of trauma. The appearance of pigmented cells at the very point of injury seemed to indicate a rôle of importance for melanophores of this fish, from the viewpoint that these cells functioned in the processes of repair and, not unlikely, in the mechanism of body defense.

As results of different experiments were found to be uniform, only a few are here reported in detail as illustrative.

Experiment 1. Goldfish, 8 cm. long from snout to base of tail, kept in still water tank, supplied by current of air. Temperature of water 78° F.

Oct. 28, 1930. Transverse incision was made with a cataract knife through a single ray of caudal fin, near the upper edge of middle part of this fin. Incision penetrated tissues over both surfaces of fractured ray.

Oct. 30. Overlying the ray near the fracture are a few scattered melanophores with irregular processes (Fig. 1). Tissues overlying the fracture are œdematous and difficult to photograph for this reason. There are a few small points of hemorrhage near the fractured fragments.

Oct. 31. A large number of melanophores, in places interlacing, surround the proximal and the distal fragment of the fractured ray as if to encapsulate the fragments (Fig. 2).

[1] Aided by grant from Blossom Fund.

Nov. 1. Active degeneration of melanophores has begun with pigment granules lying free in tissue spaces (Fig. 3).

Nov. 5. Degeneration of all melanophores in the region of the fracture, with many small pigment masses scattered throughout the field.

Nov. 8. Entire region of fracture, somewhat whitish and translucent, shows no more evidence of pigment.

In the following experiment multiple injuries were produced.

Experiment 2. Two goldfishes, 7 cm. from snout to base of tail were placed in a tank of still water fed with a current of air. The temperature of the water was gradually raised from 70° F. by heating over a period of three days to 84° F.

Sept. 23, 1930. In both fishes eight different regions were clamped with an artery forceps each for 15 seconds. The points clamped were as follows: right and left operculum, both pectoral, both ventral, the anal and the caudal fins.

Sept. 25, 2 P.M. One fish shows early pigmentation by melanophores in caudal fin, the second fish has melanophores in the right ventral fin. Pigmentation is slightly distal to crush.

Sept. 26, 3 P.M. Three days after trauma, both fishes show pigmentation by melanophores at all eight points crushed. The pigmentation is a marked one due to the large number of melanophores present in the crushed zones and neighboring tissue.

EXPLANATION OF PLATE 1

FIGS. 1–4. Experimental linear fracture by incision of a ray of caudal fin of goldfish, the injury including all tissues directly overlying fracture. Letters *A* and *B* indicate site of fracture. All photomicrographs taken from the same living fish anæsthetised with chloretone 1–2000. Magnification × 90. Temperature of water 78°–80° F.

FIG. 1. Two days after injury. A few melanophores have appeared in the œdematous tissue near the fracture, *A.B.*

FIG. 2. Three days after injury. Numerous melanophores appearing as single cells or interlacing cells at the line of fracture, *A.B.*

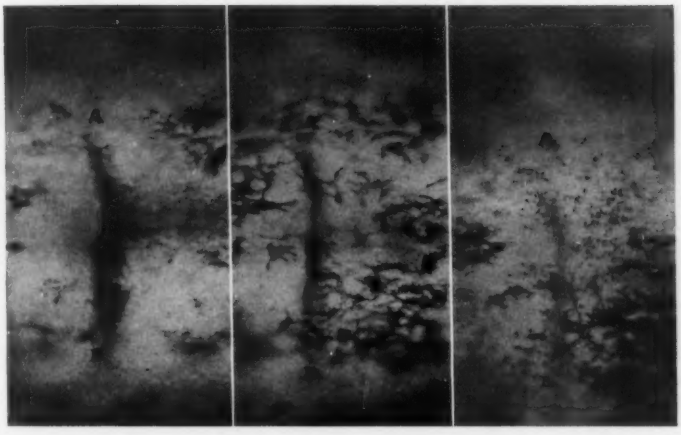
FIG. 3. Four days after injury. Degeneration of melanophores at the site of fracture *A.B.* has begun. Small black pigment masses from degenerated cells lie scattered among living melanophores.

FIG. 4. Five days after injury. Degeneration of melanophores at the site of fracture *A.B.* is complete, scattered pigment debris remains in the field. Final disappearance of all pigment on the eighth day after injury.

FIG. 5. Inter-radial tissue of caudal fin showing melanophores distributed near capillaries marked *A*, *B*, *C*, *D*. × 60. Fresh tissue removed from goldfish near an area crushed eight days previously. Fish outdoors exposed to sunlight,

FIG. 6. Irregular areas of pigmentation of melanophores developing on the surface of the body of a goldfish injured by removal of all body scales five days previously. Photograph made from living fish anæsthetised with chloretone. Size, two-thirds normal.

PLATE I



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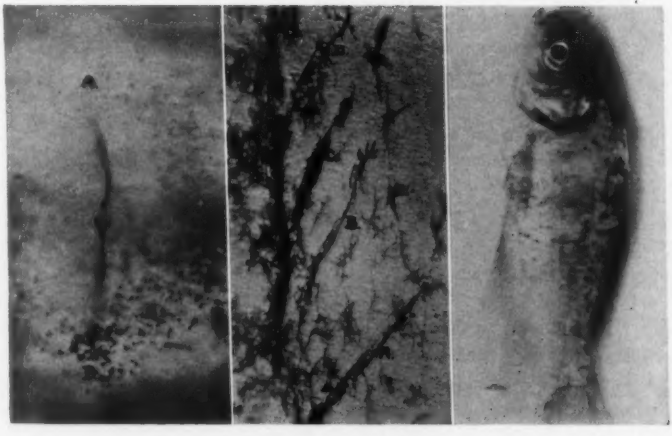

4. 5. 6.

Sept. 28. There is evidence of degeneration of melanophores at all crushed points. Temperature, 90° F.

Oct. 5. One fish is entirely clear of degenerated pigment granules at crushed point. The second shows a few black granules in the wound of the caudal fin.

Oct. 6. In both fishes all evidence of pigment formed of degenerated melanophores has disappeared at all eight points crushed. Thus these two fishes injured by crushing at eight separate points have shown, with temperature of water between 84° F. and 90° F., an intense pigmentation by melanophores at crushed points, a subsequent degeneration of melanophores, and a complete disappearance of all pigment detritus all in the course of 13 days.

Experiment 3. In this experiment, involving injury to the right operculum, 30 goldfishes, about seven cm. in length, were used. These were divided into three groups of ten. Each group was placed in a separate tank of running water in the laboratory. Fishes in Tank 1 were operated on by resecting one third of the right operculum by a straight vertical cut with scissors. Fishes in Tank 2 received a simple vertical crush for fifteen seconds of the middle of the right operculum. Fishes in Tank 3 were first crushed for 15 seconds by a clamp placed vertically in the mid-point of the operculum and all opercular tissue distal to the clamp was resected. Tank 1—Fishes (simple excision of one half of the right operculum) showed melanophores in the margin of the wound three days after operation. At first only a few such cells, but in the following two or three days there were many present. Evidence of degeneration of melanophores was noted in places as early as two days after their first appearance. Complete disappearance of black degenerated pigment from the wounded area varied between 3 and 9 days. Fishes in Tanks 2 and 3 with more severe injuries of the operculum showed a beginning accumulation of melanophores in the injured operculum also three days after trauma. The entire disappearance of pigment from the wound in fishes in Tank 2 (vertical crush of operculum) varied between 6 to 15 days after appearance of melanophores. In Tank 3 (fishes with crushed and partially resected right operculum) the eruption of melanophores at the injury occurred also three days after injury, but the final disappearance of pigmented debris varied between 9 and 16 days. One fish in Tank 1 and four fishes in Tank 2 showed slight pigmentation by melanophores of the opposite uninjured operculum, arising when the accumulation of melanophores on the injured side was well developed. Melanophores in the area of secondary pigmentation degenerated and disappeared before those in the experimentally injured right operculum.

SUMMARY

The onset of cutaneous pigmentation by melanophores in three different types of wound of the operculum carried on simultaneously in three different tanks of running water at 76° F. was uniformly between the third and fourth day after trauma. The final disappearance of pigment of degenerated melanophores of the wound area varied between 6 and 19 days after injury. In some fishes the accumulation of melanophores noted at the wound was relatively slight; in others the black pigmentation caused by large numbers of melanophores was intense and remained over a longer period.

Fishes operated on during the cold winter months and kept in tanks of cold running water (43° F.) did not show at wounded areas such a rapid development of melanophores as described in the preceding experiment. Further, pigmentation of wounds under winter temperature extended over longer periods. Thus, in nine fishes with right operculum crushed for 15 seconds with an artery clamp placed at the middle of the operculum, followed by excision of opercular tissue distal to the operculum, the following results were obtained: An eruption at the injured operculum in all nine fishes occurred between 13 to 16 days after injury; pigmentation had cleared up by degeneration of melanophores in only three fishes two months after injury, with temperature of water at 53° F. It took approximately one more month (temperature 53°–56° F.) for four more fishes to clear; the remaining two fishes cleared at the end of still another month or four months from the date of injury, when the temperature of the water had gradually risen to 61° F. The longest period of pigmentation in a wound of this series represented approximately 110 days from the date of the first appearance of melanophores.

It became of interest to learn whether or not in fishes kept in very cold water, an appearance of melanophores after trauma could be temporarily inhibited, to appear for the first time when such fishes were changed back slowly to more favorable warmer temperatures. A number of experiments were done along these lines.

Experiment 4. A goldfish, seven cm. in length, was placed in a tank of still cold water supplied by a current of air, the water varying in temperature between 42° F. and 45° F. The tank was set up in a refrigerator arranged with a double window, admitting ample daylight. It was found advisable to accustom the experimental fishes gradually to cold. By using several submerged electric lights at the beginning of the experiment and turning these off as desired, the temperature of the water could be lowered slowly without endangering the life of the fish.

Oct. 14, 1930. A small incision was made with a cataract knife in the caudal fin of this goldfish dividing transversely a single ray near the upper margin of the fin. Examination of melanophores at four day intervals negative for an entire month. Temperature 42° to 46° F.

Nov. 14. Temperature in tank raised slowly so as to reach 66° F. on Nov. 16th.

Nov. 16. Numerous melanophores appeared for the first time at fracture and along injured ray distal to this. No other black pigmentation noted.

Nov. 25. Slight pigmentation by melanophores of tip of tail and also along the margin of dorsal fin. Large accumulation of melanophores at fracture.

Nov. 27. Active degeneration of melanophores at fracture and other pigmented regions.

Nov. 29. Fish under dissecting microscope shows no pigment masses either at site of experimentally fractured ray or at the secondary points of black pigmentation of tail or dorsal fin. All melanophores have disappeared by a process of degeneration.

Fishes kept in a dark chamber, excluding all light, developed melanophores in wounds as promptly as did controls kept in daylight.

Experiment 5. Two goldfishes, seven cm. in length, with crushed right operculum and caudal fin, kept in a dark chamber in a tank of still water at 64° F., supplied by air current, were taken out of this chamber to be examined for the first time after injury on the fifth day. Many melanophores were present in crushed regions. At the same time, two control fishes, injured on the same day in a similar way, kept in a tank of equal size at the same temperature but exposed to laboratory daylight, exhibited, also for the first time, a large number of melanophores at the two crushed points. Twenty-three days after injury, one fish contained in the dark chamber and both controls were clear of pigment; the second fish in the dark chamber showed no melanophores in the injured operculum, although a few small masses of degenerated pigment masses still remained in the caudal fin.

The production of a second injury in a healed wound frequently, but not always, caused another eruption of melanophores. Refracturing a single ray at the same point, especially where the previous healing had left a wide whitish translucent area, did not produce a second crop of melanophores. The very simple injury of making a longitudinal slit in the caudal fin did not call forth melanophores either at the time of the first injury or with repeated incisions at the same point.

The irregular topographic distribution of melanophores following

trauma was seen particularly well in experiments where the scales on both sides of the body were totally removed.

Experiment 6. Nov. 1930. Three goldfishes, *A*, *B*, *C*, measuring 8, 7, 5 cm. in length respectively, kept in a heated tank of still water 76° F., supplied by air current, were operated on under chloretone anæsthesia (1–2000). All scales of the body were removed with forceps in all three fishes.

Dec. Four days after operation, melanophores appeared in irregular groups at various points on both sides of the body. The two larger fishes, *A* and *B*, showed in the course of the next few days a large number of melanophores in irregular scattered patches. The patches of pigmentation by melanophores in fish *B* are shown in Fig. 6. The smallest fish, *C*, showed only a few melanophores in small, widely-scattered areas. By the end of the twelfth day degeneration of melanophores evoked by removal of scales had occurred in all three fishes with a disappearance of broken-down pigment material. At this time (12 days after removal of scales) each fish showed definitely a set of new young scales. Fish *B* successfully withstood a second complete removal of scales, under chloretone anæsthesia, but this time only a very few rapidly degenerating melanophores developed on the denuded surface of the body, as if the supply of pigment-forming cells for these particular surface areas were partially exhausted. When, however, on the fourth day after the second operation for removal of scales the caudal fin of this fish was crushed by clamp for 15 seconds, numerous melanophores developed three days later in the crushed tail but in no other place.

Discussion and Summary

Various important problems relating to melanophores and melanogenesis appear in connection with the works of Van Rynberk (1906), von Frisch (1911), Weidenreich (1912), Asvadourova (1913), Spaeth (1913), R. Fuchs (1914), Wyman (1924), Wells (1925), Abolin (1925), Ewing (1926), Jost (1926), Bloch (1927), Cordier (1928), Becker (1930).

For the present purpose it may be of interest to recall that a number of years ago Weidenreich (1912) showed that in vertebrates the distribution of black pigment cells could be regarded as forming four distinct envelopes for the body. These envelopes he designated as "cutaneous, perineural, pericoelomatic and perivascular" respectively. He pointed out that whereas in some vertebrates several or all of these pigmentary envelopes were well developed, in other vertebrates one or more of these pigmentary envelopes might be found poorly developed, showing only a trace or rudiment of pigmented tissue. For example,

in man, where there exists a well developed cutaneous envelope of pigmented tissue, the perineural pigmented tissue is poorly developed, presenting itself as scattered black pigment cells of the piamater and elsewhere in the brain. In fishes all pigmentary envelopes are regarded as fairly well developed.

In interpreting the meaning of melanophores following injury as seen in the above experiments on goldfish, it should be kept in mind that such melanophores may represent a perivascular or perineural type of cell developing the properties of forming pigment, rather than cells belonging strictly to a system of cutaneous melanophores. It is particularly the cutaneous or corial melanophores of fishes which have received the most study to date.

Melanophores, according to Bloch (1927) show a number of morphologic peculiarities in that they form processes or dendrites and have a tendency to arrange themselves in an interlacing network. They exhibit in cold-blooded animals certain functional reactions which are shown by the spreading or the contraction of the intracellular masses of pigment granules. These reactions are changes which have their origin in nervous, actinic or hormonal stimuli; and they may also be produced by mechanical, chemical and electric means.

Ever since the description of melanophores in fishes by Siebold (1861), many investigators have contributed to the morphology of this subject. The works of Ballowitz (1912–16) on the different types of chromatophores (*i.e.*, the melanophores, xantho or erythrophores, guanophores and their various combinations forming what he designated as chromatic organs) have largely laid the basis for our present knowledge of pigment cells in fishes. This author also demonstrated histologically the innervation of melanophores in fishes.

The experimental observations of Pouchet (1876) showed a relationship between cutaneous melanophores in fishes and the sympathetic nervous system. It remained, however, for von Frisch (1911, 1912), in a series of important experiments, to demonstrate in fishes a contraction center for cutaneous melanophores in the front part of the medulla, and a secondary center in the spinal cord. Further, he explained the pathways by which impulses pass from brain through pigment motor nerve fibers to the sympathetic system and from here by means of the peripheral nerves not only to the melanophores but also to other chromatophores of the skin.

In general, the function of melanophores has been variously interpreted. In addition to the view that cutaneous pigmentation and pigment changes represent color adaptation to environment, the purpose of cutaneous pigment has been thought to lie in its protection of

deeper tissues against injurious solar rays. The migration of retinal pigment granules as it applies to vertebrates and arthropods is thought by Parker (1906) to be a mechanism calculated to protect the receptive organs of the retina from overstimulation by light and to improve the retinal images. Cutaneous pigment cells have been regarded as transforming light into heat energy. According to this view, as Weidenreich (1912) explains, the minute individual intracellular pigment granules of melanophores become heat bodies or Heizkörper, which distribute heat to neighboring protoplasm. Weidenreich (1912) has further suggested, because melanophores are innervated and react to optic, thermic and chromatic stimuli, that they may be regarded perhaps as sensory cells for color and warmth perception.

Cordier (1928) believes that the formation in cells of melanin is a process of excretion as yet not well understood. The theory implies that certain toxic waste products of metabolism gain access to special cells and there become insoluble and pigmented, their toxic products being neutralized. Elimination of pigment follows slowly as if it were a process of retarded excretion. Certain clinical cases of Addison's disease and melanosarcoma have shown melanin greatly increased in cutaneous areas and present in the blood and in the urine. This has been taken to mean a profound chemical disturbance of the body as a whole and gives support to the view that a general metabolic process may ordinarily affect the production of melanin in various regions of the body.

Whatever may be the relationship to the nervous system of melanophores resulting from trauma as seen in the present experiments on goldfish, it seems plausible from their structural arrangement in healing wounds, that such melanophores are pigmented cells which function in repair of damaged tissue. Melanophores of this kind appeared relatively early in the course of wound-healing when favorable warm temperatures were employed. They disappeared by a process of degeneration at the site of the wound when healing proceeded and usually when the covering of the wounded surface was nearing its completion. Whereas melanophores showed in wounds of goldfishes within 3 or 4 days after injury when fishes were kept in water of relatively warm temperature (70°–90° F.), with fishes kept in cold water (40°–42° F.) the appearance of melanophores in wounds was retarded or even inhibited, to appear for the first time when these fishes were returned to a warm environment. A temperature of 40° F. was found sufficient to inhibit the appearance of melanophores for a month.

Fishes kept in a dark chamber completely excluding light showed melanophores in various experimental wounds as early as did controls

kept under usual laboratory conditions exposed to light. Fishes kept in tanks out-of-doors and in this way exposed directly to the sunlight developed melanophores in wounds a few days later (Fig. 5). The reaction here seemed intense. In some of these fishes melanophores developed not alone at the crushed points, but also in areas adjacent to the wound and in all other fins.

When studied in a simple form of injury such as dividing transversely a single ray of the caudal fin, melanophores appeared first as periadventitial cells in close relation to the outer walls of the small capillary blood vessels which covered the surface of the ray near the fracture. With an increase in numbers, the melanophores spread toward the region of the fracture and formed a network (Fig. 2) in the corium by the interlacing of the numerous irregular processes. Degeneration in individual melanophores was observed as early as 24 hours after their first appearance near a fractured ray. Fixed paraffin sections of tissue with degenerating melanophores showed a moderate number of phagocytic cells containing pigment. For the most part, however, the impression was gained that the pigment detritus rested free in the tissue spaces preparatory to removal by lymphatics, or became dissolved *in situ*.

The actual production of melanin in cells is now generally regarded as the result of enzyme action. The important studies of Bloch (1927), advancing the views on the intracellular production of melanin by enzyme, are too well known to need repetition here. It is conceivable that in the experimental wounds of goldfish chemical changes occur locally permitting melanin to be formed in periadventitial cells irregularly distributed in the corium of the injured area.

Experimental wounds of goldfishes quite naturally are constantly open to infection by bacteria or parasites. Numerous bacteria and especially cocci were seen in paraffin sections of tissue from crushed operculum at various stages after injury before complete healing had occurred. When, as occasionally noted, a growth of fungus appeared in connection with experimental wounds, pigmentation by melanophores appeared particularly intense, affecting not alone the wound but also adjacent areas. There was at times pigmentation of the fins other than the ones experimentally injured and, in rare instances, a patchy pigmentation of body scales under these circumstances. Treating such wounds for several days in succession with two per cent mercurochrome destroyed the parasites, and pigmentation of the wound with secondary pigmented areas then disappeared. The presence of bacteria in wounds and the large number of melanophores present in injured areas affected with parasites, suggest a possible rôle for melanophores in the mechanism of body defense.

Goldfishes subjected to a total removal of scales showed in the course of several days a distribution of melanophores varying in extent and intensity in different fishes. This eruption was asymmetrical, irregular and patchy, as if periadventitial cells capable of forming black pigment as a result of trauma or during subsequent wound regeneration, actually occupied a very irregular distribution on both sides of the body. As new scales formed in these experimentally produced scaleless fishes, melanophores disappeared by degeneration. A second total removal of scales in one of the fishes was followed by a very scanty eruption of melanophores, as if the possibility of local melanophore production in this instance were, temporarily, at least, exhausted.

Usually, but not always, a re-injury at the same point brought out a second eruption of melanophores differing but little from that which followed the primary injury.

The eruption of melanophores in experimental wounds of the goldfishes, varying in intensity in different fishes, appears to indicate that such melanophores, probably periadventitial in origin, form in response to injury and function in the repair of injured tissues.

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STUDIES ON THE INTESTINAL FLORA OF TERMITES WITH REFERENCE TO THEIR ABILITY TO DIGEST CELLULOSE

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Introduction

Interest for a considerable time has been centered on the ability of certain organisms to derive nourishment from a wood diet, the principal constituents of which are cellulose and lignin, both resistant to the digestive action of enzymes normally present in the digestive tract of most animals. Animals such as termites, larvæ of wood-boring beetles, and a bivalve, the shipworm *Teredo navalis*, so injurious to ships and piles, have been conspicuous for their ability to digest cellulose. In the case of *Teredo navalis* (Dore and Miller, 1923), digestion has been explained by the production of cellulose-digesting enzymes. The explanation in the case of termites is a more interesting one.

Microscopic examination of the intestinal content of most species of termites discloses countless numbers of Protozoa and bacteria. Careful experiments have been carried out to explore the relationships between the host and their intestinal organisms, and it has been shown conclusively that the termites are absolutely dependent upon the Protozoa present for the digestion of the cellulose in their food.

L. R. Cleveland, one of the foremost workers in this field, has shown that termites containing an intestinal fauna of Protozoa were able, under favorable conditions, to exist on a diet entirely made up of Whatman filter paper of the purest grade, and he successfully reared them upon this diet for over two years. If, however, he defaunated the termites (by incubating them at 36° C., the Protozoa were killed, but the termites were unharmed), they were unable to live upon the pure cellulose and soon died. If he re-inoculated the termites with Protozoa, however, after incubation, they were able to live indefinitely upon the filter paper (Cleveland, 1924).

Biological Considerations

Comparatively little work has been done with respect to the biological relationship between termites and the other intestinal organisms usually present. Hollande (1922) discusses the morphology and

reproduction in a considerable number of spirochætes which he found very abundant in the intestinal contents. Hoelling (1910), in his paper on "The Nuclear Conditions of Fusiformis termitidis," describes the morphology of fusiform bacilli studied from a number of smears of the intestinal content of termites. Imms (1924) states that "Portier investigated an apparent symbiosis in the case of the larva of *Nonagria* which lives within the stems of *Typha* devouring the pith. . . . In the digestive tube of this larva are found great numbers of motile conidia of a fungus (*Isaria*), which exist among the devoured vegetable fragments. The conidia are always accompanied by a micrococcus which secretes an enzyme capable of dissolving cellulose. Portier states that the conidia develop at the expense of the dissolved cellulose and eventually penetrate the walls of the gut, escaping into the blood. Most of them are there attacked by phagocytes and transformed into products which serve to nourish the tissue of the host."

In an attempt to determine whether the Protozoa were entirely responsible for the digestion of cellulose in the digestive tract of termites, Cleveland (1924) studied the bacterial flora of *Reticulitermes flavipes*. He states that bacteria were sometimes numerous, and he attempted all known methods, aërobic and anaërobic, for isolating cellulose-digesting bacteria. One hundred attempts were made, but all results were negative, even after the cultures were more than two months old. Ten attempts to isolate cellulose-decomposing molds and actinomycetes were made and were unsuccessful. In an attempt to isolate the organisms, an inorganic medium was made containing:

K_2HPO_4	1.00 gram
$MgSO_4$	0.50 gram
KCl	0.50 gram
$FeSO_4$	.01 gram
$NaNO_3$	2.00 grams
H_2O	1000 cc.

To this medium cellulose was added in two forms: a small piece of Whatman's filter paper and 0.5 per cent cellulose suspension. To the inorganic medium containing cellulose suspension sufficient agar was added to make a solid medium. Incubation was apparently 36° C.

Cleveland (1928) observed that all families of termites harbored many spirochætes which he thought might play a rôle in the digestion of cellulose and hemicellulose. He observed millions of these, often attached to a single Protozoön, and easily mistaken for flagella. Attempts to grow the spirochætes failed and animal inoculations proved negative. By feeding the termites cellulose thoroughly moist-

ened with a 5 per cent aqueous solution of acid fuchsin, he found it possible to remove in this manner all spirochætes without doing any damage whatever to the Protozoa or to the termites. He concluded that the spirochætes play little if any rôle in the digestion of wood and cellulose.

In the wood-ingesting larvæ of certain insects, characteristic blind sacs and diverticula of the digestive tract have been demonstrated in which, aided by the action of myriads of bacteria, food particles are held and digested. From the larvæ of rose-chafers (*Potosia cuprea*) cellulose-digesting bacteria have been isolated in pure culture. These slender peritrichiate, anaërobic rods (*Bacillus cellulosae fermentans* Werner) are found free also in the ant hills inhabited by *Potosia cuprea*. The optimum temperature for fermentation was found to be 33–37° C., the minimum 21° C. The larvæ are so dependent upon these intestinal organisms that the increase in weight of the larvæ is determined by the temperature. If the temperature of the ant hill at the end of October goes below 21° C., the now useless taking-in of food material is suspended (Buchner, 1928).

Experimental

a. The Food of Termites

It was the purpose of the present experiment to determine whether cellulose-digesting organisms occurred in woody material upon which termites feed. The woody material from termite colonies was first examined for cellulose-digesting organisms. Some material obtained from a termite colony in March, 1929, which had been kept in a dry condition since that time (almost one year), was used to inoculate nitrate-cellulose tubes. The material contained wood particles, termite excreta and a small quantity of fine reddish clay.

The nitrate-cellulose medium was made according to the formula of Bradley and Rettger (1927). It contained:

Di Potassium phosphate	1 gram
Magnesium sulfate	1 gram
Sodium chloride	1 gram
Calcium carbonate	2 grams
Potassium nitrate	2 grams
Distilled water	1000 cc.

The cellulose was provided in the form of strips of filter paper. Tubes were kept at room temperature and incubated at 34.5° C. and 60.5° C. under aërobic and anaërobic conditions.

Ten tubes were incubated at room temperature under aërobic

conditions. In every one of these there was abundant growth and discoloration of paper above the liquid level. Marked discoloration appeared in all tubes in four days. Seven tubes labeled G–1 to G–7 inclusive were inoculated from two of the above tubes on March 1, 1930. When examined on March 3, 1930 discolored areas were present on the paper of all tubes. On the original tubes the discolorations were yellow-green, yellow and light brown. On the "G" tubes the predominant color was dark brown.

Three tubes inoculated and placed under anaërobic conditions on February 19, when examined on March 8, showed no marked discoloration and no cutting of paper at this time. (Anaërobic conditions were produced in a Mason jar, using pyrogallic acid and sodium hydroxide.) On this date the three tubes were placed under aërobic conditions. When examined April 7, tube no. 16 showed cutting of paper at liquid level and maceration of paper below liquid level. Mold growth apparently was inhibited.

A tube no. 12 incubated at 34.5° C. under aërobic conditions on February 19, when examined on February 25 showed marked discoloration similar in variety and extent to that produced at room temperature. The paper, however, was not cut at liquid level.

Three tubes 17, 18, and 19, inoculated and incubated at 34.5° C. under anaërobic conditions, showed no noticeable discoloration or cutting of paper when examined 18 days later. The tubes were removed from the incubator and placed under aërobic conditions, at room temperature. When examined 16 days later, the paper in tubes 18 and 19 was entirely cut at liquid level with no characteristic discoloration. The paper below the liquid level was entirely macerated. (The paper was probably cut before the day of examination.)

A tube incubated at 60.5° C. under aërobic conditions showed, when examined about 10 days later, a few small, isolated areas of growth on paper.

Three tubes were inoculated and incubated at 60.5° C. under anaërobic conditions on February 20. When examined on March 7, one of the tubes showed paper cut at liquid level and macerated at lower portion below liquid level. In this tube the paper was so macerated that upon slight shaking it fell apart into loose fibers below liquid level.

From this last tube three tubes were inoculated and incubated at 60.5° C. under aërobic conditions on March 8, three silica-gel plates inoculated from the above tube also, were incubated anaërobically at 60.5° C. On April 7, neither plates nor tubes showed discoloration or growth. Re-inoculated nitrate-cellulose tubes at 60.5° C. under anaërobic conditions showed no growth after one month.

Woody material obtained with termites from Dr. Cory was used to inoculate a tube at room temperature under aërobic conditions. The material was composed of wood and digested material, but no loose soil. When examined 9 days later the paper at the liquid level showed a discoloration which was marked above liquid level. Green discoloration was prominent. One colony 0.5 mm. in diameter produced a wine-colored discoloration. Transplants on silica-gel plates produced abundant growth.

The silica-gel was made fundamentally according to the general formula of Winogradsky (1929). Seventy-five grams of potassium silicate were dissolved in 1000 cc. of distilled water. To this was added an equal quantity of HCl of a specific gravity of 1.10. Thirty cc. of mixture were placed in the petri dishes and set aside under cover for 24 hours. They were then immersed in running water for 24 hours. They were next washed four successive times for 24 hours each in large covered dishes of sterile distilled water. From the following inorganic salt solution (made up for 100 plates) 2 cc. were added to each silica-gel dish.

KH_2PO_4	1.0 gram
$MgSO_4$	0.5 gram
NaCl	0.5 gram
$FeSO_4$	0.01 gram
$MnSO_4$	0.01 gram
KNO_3	3.6 grams
$CaCO_3$	2.0 grams
Distilled water	200 cc.

The pH was adjusted to 7.2. The petri dishes were then placed in an incubator at 60.5° C. until excess moisture evaporated. Sterilized pieces of Whatman's filter paper were placed aseptically on silica-gel plates. Sterile covers were then placed over the plates. Stroke inoculations were made.

Termites received from Dr. Cory were transferred to clean petri dishes in which were placed filter paper and the tissue paper sent with the original shipment. This was being eaten by the termites. On March 8, pellets of termite excreta which had been dropped on the tissue paper were used to inoculate two nitrate-cellulose tubes and one silica-gel plate, and kept at room temperature. (The pellets were clean and the color of tissue paper.) On March 10 no visible growth was evident in the tubes. Yellow discoloration was noticeable on the paper around the pellets in the silica-gel plates. This growth later covered the entire paper and the paper showed almost entire digestion by May 10. Nitrate-cellulose tubes inoculated from this plate showed growth

and digestion of paper in 5 days. A gelatinous milky-white growth with translucent areas developed on paper. Microscopic examination showed maceration of fibers with attached bacteria. The growth was characterized by the presence of mold growth which formed the gelatinous milky mass.

b. The Intestinal Contents of Termites

Most of the experiments with the intestinal contents were carried out with termites of the genus *Reticulitermes* collected at Mullica Hill, New Jersey, and with *Termopsis*, received from Dr. Kirby, at the University of California.

Reticulitermes.—Microscopic examination of intestinal contents showed besides the myriads of Protozoa, spirochætes ranging in length from 5 to 15 μ; the smaller ones were in great abundance, and apparently more numerous than the Protozoa. Motile rods and filamentous rods were also present. Examinations were made with hanging drops in physiological salt solution and from smears stained with alcoholic fuchsin.

On March 31, seven inoculations were made with intestinal contents of seven termites, washed 4 minutes in 1–1000 $HgCl_2$, then rinsed with sterile distilled water. The intestinal contents were squeezed out with sterile forceps onto silica-gel plates. These were kept anaërobically at room temperature. On April 1, sixteen termites were used as above to inoculate silica-gel plates. Eight of these were kept under anaërobic conditions and eight were kept at room temperature under aërobic conditions. On April 2, twenty termites were used as above to inoculate silica-gel plates kept at room temperature under aërobic conditions. On April 1, six termites were used to inoculate six nitrate-cellulose tubes.

When the above cultures were examined on May 12, none of the anaërobic plates showed growth. Of the aërobic silica-gel plates eight inoculations showed a slight mold growth, with no cutting of paper and no distinct discoloration. The remaining inoculations on plates produced no growth. One of the six nitrate-cellulose tubes showed clearly digestion of paper, with translucent areas, and microscopic examination of paper from this tube showed numerous bacteria, 1×0.75 μ in size on the fibers.

Four tubes inoculated on February 28 with *Reticulitermes flavipes* received from Dr. L. R. Cleveland showed no growth on March 10, and no growth was visible on April 30.

Termopsis.—The specimens were large enough to enable one to dissect out the digestive tract with sterile forceps. All termites were

first washed for 5 minutes in 1–1000 $HgCl_2$ and then rinsed in sterile distilled water. All inoculations were made on silica-gel plates, which were kept under aërobic conditions at room temperature.

On April 21, eight termites were used for inoculations. On April 23, two winged termites were used to inoculate silica-gel plates. On April 24, ten winged termites were used for inoculations. On April 24, eight worker termites were used as above. On May 2, six workers were used as above. On May 2, eight winged forms were used as above. On April 23, ten termites were used to inoculate silica-gel plates which were kept under anaërobic conditions. When examined on May 17, none of the plates showed cellulose-digestion or indication of growth of cellulose-digesting organisms. There was mold growth on intestinal contents of eighteen termites. The growth of molds was evidently due to the fact that treatment for 5 minutes with 1–1000 $HgCl_2$ did not kill them.

Beckwith and Rose (1929) obtained cellulose digestion in a number of cases when working with intestinal contents of termites, but their results cannot be taken as conclusive, since they attempted to sterilize the termites externally by merely washing them in tincture of iodine (U.S.P.) for 45 seconds. This short exposure and the fact that small air bubbles captured between the hairs of the insect would prevent contact with the germicide in this time, would indicate that the organisms on the surface were not destroyed.

Discussion of Results

Cleveland and others have shown that digestion of cellulose in termites is entirely dependent upon intestinal organisms. Cleveland has shown that termites are not dependent upon intestinal spirochætes, although he has not shown that the spirochætes do not play an important part in cellulose digestion.

In every experiment carried out in the present study with woody material from termite nests, abundant growths of cellulose-digesting organisms were obtained. The numerous cellulose-digesting organisms, which were undoubtedly taken into the digestive tract with food, could not again be isolated from the intestine on the cellulose media used.

The most conspicuous organisms, with reference to numbers and bulk next to the Protozoa, as seen by microscopical examination of the intestinal contents of termites, are the spirochætes. Since these do not grow on the usual laboratory media their true significance has not been explained.

Summary and Conclusions

Cellulose-digesting organisms, both bacteria and molds, are very abundant in termite nests. These organisms have been obtained in abundance from material dried for a year.

Cellulose-digesting bacteria were not isolated from the intestines of termites on the nitrate-cellulose medium of Bradley and Rettger nor on the silica-gel medium of Winogradsky.

True bacteria are probably of little importance in cellulose digestion in termites.

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THE INNERVATION OF THE STOMACH AND RECTUM AND THE ACTION OF ADRENALINE IN ELASMOBRANCH FISHES

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A study of the literature concerning the innervation of the stomach and intestine in mammals reveals much confusion and contradiction. The orthodox differentiation into sympathetic and parasympathetic with antagonistic actions has many exceptions. Thus Langley (1898) found inhibitory fibers to the stomach in the vagus of the rabbit, Morat (1893) found excitatory fibers to the stomach and intestine in the splanchnic of the dog, and Carlson, Boyd and Pearcy (1922) have found that both the splanchnics and the vagi of the cat carry both kinds of fibers to the stomach. On the basis of effects produced by adrenaline, Smith (1918) assumed the splanchnics to be inhibitory for the stomach in man and in the cat, but only for certain parts of the stomach in the guinea pig, rabbit and dog, while being motor for other parts. Tashiro (1920), however, using adrenaline on surviving cat intestine, came to the conclusion that there are motor fibers to the circular muscle in the sympathetic nerves as well as inhibitory fibers to both the circular and the longitudinal layers. McCrea, McSwiney and Stopford (1925) found that in dogs, cats and rabbits the primary effect of stimulation of the peripheral cut end of the vagus on the stomach may be inhibition or augmentation, depending upon the intragastric pressure, but that the final effect is motor. Brown, McSwiney and Wadge (1930) found that the effect of sympathetic stimulation depends on the type of stimulation. A low frequency contracted the body of the stomach in the cat, whereas ordinary tetanizing current inhibited. All rates inhibited the antrum, and adrenaline caused an inhibition of both parts. In a review Van Campenhout (1930) says, "We believe the actual distinction of sympathetic, parasympathetic and local innervations to be erroneous owing to ignorance of the real constitution of the visceral autonomic nervous system." A similar view was expressed by Langfeldt (1929), who concluded that there is no absolute antagonism between the sympathetic and parasympathetic and that our information concerning the peripheral termination of both systems is incomplete.

The literature concerning the visceral innervation in the lower vertebrates shows no more conformity to the orthodox view than does that in mammals. Goltz (1872) showed that the splanchnic nerves are motor for the stomach in the frog, and this has been confirmed by Dixon (1902), Müller and Liljestrand (1918) and Itagaki (1930). Dixon (1902) found the vagus in the frog to have either an inhibitory or a motor effect on the stomach. For reptiles there is not enough evidence to make a comparison, either anatomical or physiological, although Thorell (1927) by the use of adrenaline considered the sympathetic to be inhibitory to all parts of the turtle's stomach except the cardiac portion. In birds Nolf (1925) has reported that the vagus is motor to the crop, and either motor or inhibitory to the gizzard and small intestine; and the coeliac nerves are either motor or inhibitory to the gizzard and duodenum.

In elasmobranch fishes the autonomic nervous system appears not to be well developed (Müller and Liljestrand, 1918). Bottazzi (1902) found both the vagus and the anterior splanchnic nerves in *Scyllium canicula* to be motor for the stomach. He was unable to demonstrate any inhibitory effect of either. Stimulation of the cord in the region from the forty-fifth to forty-eighth spinal nerves gave motor activity of the rectum. Müller and Liljestrand (1918) confirmed Botazzi (1902) in part, using *Squalus acanthias* and various species of *Raia*, but believed an inhibitory effect of the vagus on the stomach to be more marked than the motor effect. They never obtained evidence of inhibition from the anterior splanchnics. Stimulation of the middle and posterior splanchnic nerves was without effect on the spiral valve and rectum.

In view of other peculiarities of the autonomic nervous system in elasmobranchs, namely, the lack of accelerator nerves to the heart (Bottazzi, 1902; Müller and Liljestrand, 1918; Lutz, 1930*a*) and the inhibitory action of adrenaline on the heart (Macdonald, 1925; Lutz, 1930*b*), the present writer believed that it might prove useful to compare the effects of adrenaline and extract of chromaphil tissue on parts of the gut with the effects of electrical stimulation of the extrinsic nerves to the same parts.

Material and Method

The elasmobranchs used were *Squalus acanthias*, *Raia erinacea* and *R. diaphanes*. For anatomical reasons only *S. acanthias* was used when nerves were stimulated. Segments of the stomach, spiral valve, and rectum one half to one inch long were suspended in 50 cc. of a physiological solution described by Lutz (1930*b*), and tracings ob-

tained. Some pieces were hung so that the circular muscles would activate the lever, others were suspended so that the longitudinal layer would be most effective. Adrenalin chloride (Parke, Davis & Co.) and chromaphil tissue extracted in distilled water were added to the bath by means of a pipette. Control tests, in which similar amounts of distilled water and extracts of liver and spleen were added to the bath, showed that the method was satisfactory. An extract of the anterior chromaphil bodies was made in one cc. of distilled water immediately on removal of the tissue, and used at once. In a few cases, in which *R. stabuliforis* served as a source of chromaphil tissue, only one "axillary heart" was used to one cc. of distilled water,

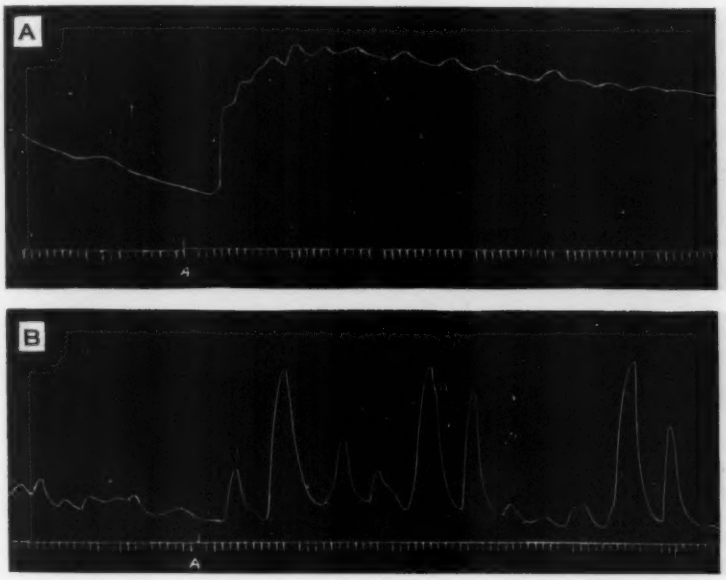

FIG. 1. Effect of adrenalin chloride, 1 in 50,000, on the pyloric portion of the stomach. Time in 5 second intervals. *A*, *Raia erinacea*. Typical effect on tonus. *B*, *R. diaphanes*. Effect mainly on motility.

but in the case of the smaller species of *Raia* both anterior chromaphil bodies were used and sometimes, in addition, some of the accessory bodies.

In the experiments in which the extrinsic nerves were stimulated the entire central nervous system was pithed. The left vagus was exposed through the anterior cardinal sinus. The first sympathetic

ganglion (gastric) and the anterior splanchnic nerves were exposed through the posterior cardinal sinus, or the latter were sometimes stimulated along the course of the coeliac artery. The posterior splanchnic nerves (rectal) were stimulated along the posterior mesen-

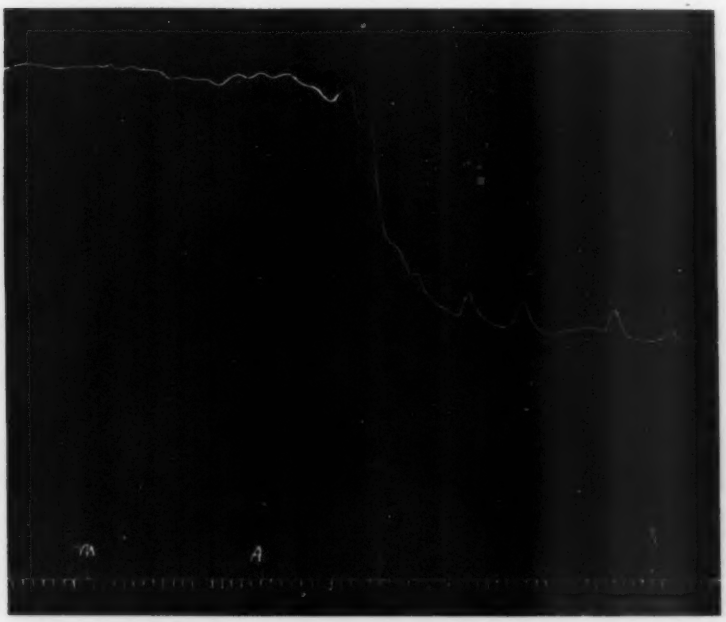

FIG. 2. Effect of adrenalin chloride, 1 in 50,000, on the rectum of *Squalus acanthias*. Time in 5 second intervals. At *M*, 2 cc. of the bath fluid squirted on the preparation. Adrenalin chloride added at *A*.

teric artery or in the mesentery supporting the rectal gland. Records of the movement of the gut were obtained by means of a small hook and a light lever. The nerves were stimulated with faradic current by means of platinum electrodes leading from an inductorium (Harvard Apparatus Co.) with the secondary coil set at 8 cm. and one 2.5 volt dry cell in the primary circuit.

RESULTS

Adrenalin chloride added to the bath fluid to make one in 50,000 caused a rise in tone and sometimes augmentation of rate and height of the movements of the pylorus and other parts of the stomach in twenty-five preparations and had no effect in three cases (Fig. 1).

In some inactive preparations motility was initiated by a similar dose. A distilled water extract of chromaphil tissue taken from the skate gave the same effect as adrenalin chloride on the pylorus and stomach of both the skate and the dogfish (Fig. 3). Extracts of liver and spleen, agitation of the bath fluid, or the addition of distilled water gave no response.

On twelve preparations of the posterior end of the spiral valve and the rectum, adrenalin chloride, one in 50,000 caused a marked fall in tone and an inhibition of motility (Fig. 2). In no case was

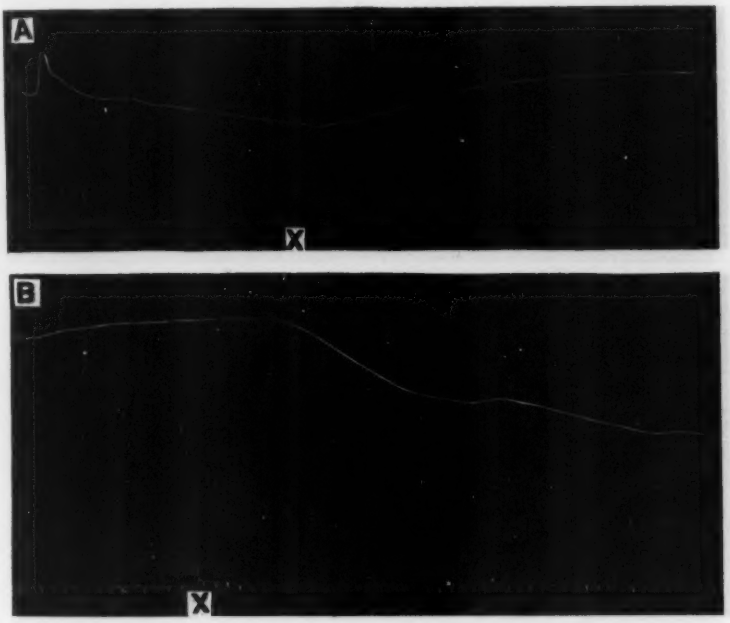

FIG. 3. Effect of extract of chromaphil tissue. Time in 5 second intervals. *A*, *Squalus acanthias*, pyloric portion of the stomach. *X*, extract of the axillary bodies from one side of *Raia stabuliforis*. *B*, *R. erinacea*, rectum. *X*, extract of the axillary bodies of the same specimen.

there activation or failure of response. Extract of chromaphil tissue also caused inhibition (Fig. 3). Extract of liver gave no response.

In thirteen specimens of *S. acanthias* faradic stimulation of the first sympathetic ganglion (gastric) or the anterior splanchnic nerves caused extensive contractions of the stomach beginning in the pyloric region. The latent period varied from two to five seconds (Fig. 4, *A*).

In seven fishes faradic stimulation of the peripheral end of the cut vagus at the anterior cardinal sinus caused moderate contractions of the pylorus and adjacent region. The latent period was about five seconds. In one case no response was obtained. The response from the vagus was never obtained longer than forty minutes after the

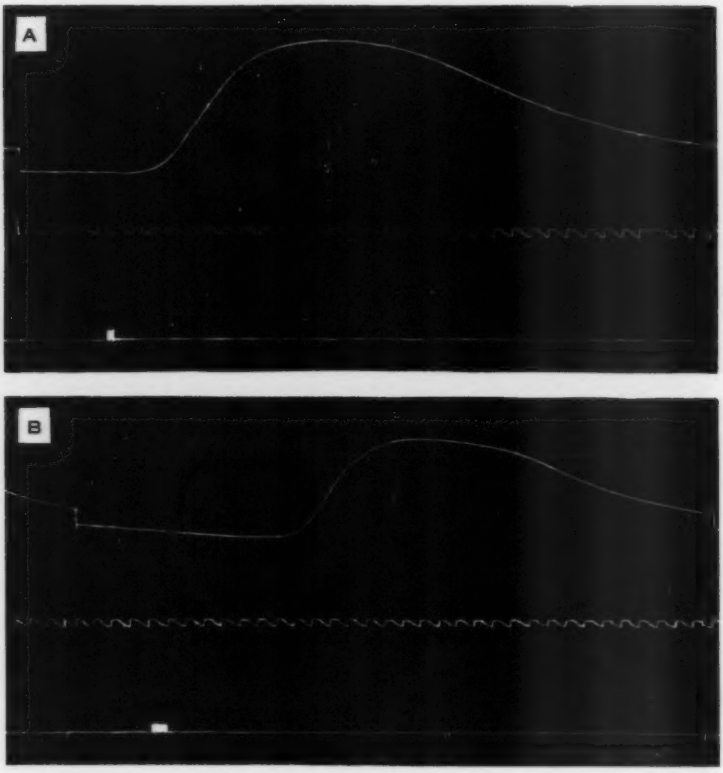

FIG. 4. Effect of faradic stimulation. Time in seconds. *A*, *Squalus acanthias*. Contraction of the pyloric portion of the stomach on stimulating the first sympathetic ganglion (gastric) for one half second. Latent period, 2.5 seconds. *B*, *S. acanthias*. Contraction of the rectum on stimulating the posterior splanchnic nerves for one second. Latent period, 8 seconds.

opening of the cardinal sinuses, whereas the sympathetic response was obtained after three hours.

In four fishes the posterior splanchnic nerves were stimulated and in each case a vigorous contraction of the rectum and the adjacent

part of the spiral valve was repeatedly obtained (Fig. 4, *B*). The response had a latent period of eight to ten seconds, and in one specimen was active after three hours without the circulation.

Discussion

The motor effect of electrical stimulation of the sympathetic and of adrenaline on the stomach of the elasmobranch is another exception to the view that in general the sympathetic is inhibitory to the gut and the vagus motor. In this case the effect of adrenaline is sympathico-mimetic. If the posterior splanchnic nerves, stimulation of which activates the rectum, are sympathetic, as Müller and Liljestrand (1918) describe them to be, then the inhibitory action of adrenaline on this part of the intestine is also an exception. Brown, McSwiney and Wadge (1930) found that adrenaline did not reproduce the effects of sympathetic stimulation of the stomach in the cat and in the dog, inhibition being the invariable result.

The results reported here confirm Bottazzi (1902) working on *Scyllium*, and Müller and Liljestrand (1918) working on *Squalus* and *Raia* insofar as the effect on the stomach of electrical stimulation of the anterior splanchnic nerves is concerned. However, since a marked contraction of the rectum resulted from stimulation of the posterior splanchnic nerves, and no evidence of inhibition of the stomach through stimulation of the vagus was obtained, these results are to that extent at variance with those of Müller and Liljestrand.

While there may be a valid reason for perpetuating the morphological division of the autonomic nervous system into cranial, thoracolumbar and sacral parts, there is sufficient evidence to indicate that a general physiological distinction should not be made so far as control of the alimentary tract is concerned.

Summary

1. Adrenalin chloride and extract of the chromaphil bodies caused a rise in tone and sometimes an increase in motility of all parts of the stomach of *Squalus acanthias*, *Raia erinacea* and *R. diaphanes*.

2. Faradic stimulation of the first sympathetic ganglion (gastric) and the anterior splanchnic nerves caused extensive contractions of the stomach beginning at the pylorus in *Squalus acanthias*. Similar stimulation of the vagus caused moderate activity in the region of the pylorus.

3. Adrenalin chloride and extract of chromaphil bodies caused a marked decrease in tone and inhibition of motility of the posterior end of the spiral valve and the rectum in all three elasmobranchs.

4. Faradic stimulation of the posterior splanchnic nerves caused a vigorous contraction of the rectum and adjacent part of the spiral valve in *Squalus acanthias*.

5. The data presented here and the evidence from the literature indicate that a general physiological distinction between the sympathetic and the parasympathetic divisions of the autonomic nervous system should not be made.

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LABORATORY REPRODUCTION STUDIES ON THE GROUND SQUIRREL, *CITELLUS TRIDECEMLINEATUS PALLIDUS*, ALLEN[1]

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INTRODUCTION

In studies on hibernation carried on in this laboratory for the past six years hundreds of ground squirrels, *Citellus tridecemlineatus*, have been kept in our animal house. During this time many pregnant females have been received in the spring and have usually reared their young, but mating has been known to occur in the laboratory only in two females, both *C. t. pallidus* Allen,[2] the variety used in these experiments. Since the animals were well cared for and were in good health, it seemed desirable to investigate the possible causes of their sterility for the scientific as well as the practical information which such a study might yield. It was expected that reproduction was unlikely to occur except at about the time of the normal breeding season in April and May (Drips, 1919; O. Wade, 1927), but why rut is limited to the spring in this and many other species is another question which studies of the present type may in time help answer.

For the sake of brevity the specific methods and the literature will be considered with the different types of experiments. The majority of the animals in the laboratory served as controls for the special experiments. These controls were kept in wire cages with wood bottoms measuring two by three feet. From one pair to about six animals were usually kept in one cage. Wood shavings were used on the floor and small wooden boxes in which the animals could build nests were usually provided.

OBSERVATIONS ON CONTROLS

Since our ultra-violet light, outdoor cage and ovarian extract experiments were performed between March and June, 1930, inclusive, a group of 6 males and 11 females were observed as special controls

[1] Contribution No. 132 from the Department of Zoölogy, Kansas State Agricultural College, Manhattan.

[2] As these animals were secured in central western Kansas it is possible that some of them may have been *C. t. arenicola* the southern variety recently split off from *C. t. pallidus* by Howell (Proc. Biol. Soc. Wash., 41: 213, 1928).

during these months. In three of these males the testes were partly enlarged and had migrated from the abdomen, the position during the fall and early winter, partly into the scrotum which was enlarging. The latter condition of enlargement of the testes and descent into the scrotum, which becomes darkly pigmented, is typical of the breeding season and animals in this condition will be referred to as "scrotal." Animals showing partial enlargement of the testes and partial descent without enlargement of the scrotum will be called "partly scrotal." In the eleven control females only one had the vagina open between March and June.

As the pituitary implantation experiments and one ovarian extract experiment were not limited to the spring, it is important to refer to the sexual development over a longer period than that given for the special controls already mentioned. For years it has been observed that captive male ground squirrels became "scrotal" in late winter and in the spring, some being in this condition when others were not. Several females had also been seen with swollen and even open vaginæ. While the observations from March to June, 1930 showed only a small proportion of animals with enlarged external genitalia, probably because of the lateness, observations made in 1931 with the assistance of Mark A. Foster showed a pronounced development in January and February. In fact a majority of males were "scrotal" January 10. The females, with one exception, showed no external swelling on this date. By February 1 all of the healthy males (26) were scrotal and nine of twelve females more than one year old had very swollen vaginæ, and three of the nine had the vagina open. Of the females which were less than one year old, seven of sixteen had very swollen but not open vaginæ.

Since males and females have nearly always been together in our laboratory there should have been ample opportunity for a great many to breed each year, but none of the animals which have been in the laboratory over winter have yet been known to breed, and only two cases of reproduction among newly received ground squirrels are on record in this laboratory. One female received May 1, 1930 gave birth to a litter on June 22, 1930, and therefore must have bred in the laboratory about May 25. In 1926 one case of breeding in the laboratory had been witnessed on April 24, the day the animals were received. The litter was born about 27.5 days later. This is in agreement with data for *C. t. tridecemlineatus* by Drips (1919), who gives the period of gestation as 28 days, and by O. Wade (1927), who reports two cases of reproduction in the laboratory in which it was between 27 and 28 days.

Nutrition and Vitamins

The animals were fed a ration which we had found to maintain good health and reproduction in rats and mice. It consisted of yellow corn meal (30 per cent), whole wheat flour (30 per cent), skim milk powder (30 per cent), alfalfa meal (4 per cent), bone meal (4 per cent), salt (1 per cent), and cod liver oil (1 per cent). The cod liver oil was added just before feeding. Sprouted oats or green feed and water were also supplied.

The well nourished appearance of the animals showed this diet to be adequate for health. Vitamin A, which may have a slight influence on reproduction (Sure, 1928; Evans, 1928*a*), was present in the cod liver oil, wheat and corn. Vitamin B, whose absence might produce a poor physical state with loss of reproductive power in the male (Evans, 1928*b*; Mattill, 1927) or cessation of œstrus in the female in four weeks, with death some two months later (Parkes, 1928), was supplied by the wheat, corn and alfalfa. Vitamins C and D apparently have little effect on reproduction but they were supplied, C by the green feed and D by the cod liver oil, wheat and green feed. An absence of vitamin E would produce a degeneration of seminal epithelium in the male and an early resorption of the young in the female (Evans, 1925; Evans, Burr and Althausen, 1927), but this vitamin was supplied by the wheat, corn, alfalfa and green feed. The addition of wheat "germ stock" which is especially rich in vitamin E, to the diet of 24 animals through the month of June, which would be the latter part of the normal breeding season, did not affect genital development or reproduction.

Pituitary Implants

The work of Smith (1927*a*, *b*) and Smith and Engle (1927) has shown that implantation of the anterior lobe of the pituitary may produce precocious sexual maturity and super-ovulation in mice and rats. These authors have shown that similar results are obtained from implants of the whole gland, indicating that the inclusion of the posterior lobe does not affect the action of the anterior lobe in such implants. In attempting to stimulate the reproductive organs of the ground squirrels, implants of pituitary glands from rats were made into adult ground squirrels. While the entire gland was used, the results obtained should be attributed to the anterior lobe alone as already indicated. About thirty animals were used from November to August. Those used during the summer had been in the laboratory over winter and therefore were at least one year old. A finely cut pituitary from a rat was implanted or injected, with a small amount of physiological salt

solution, subcutaneously in the hind leg of the anæsthetized animal by means of a fine glass canula. A striking external genital enlargement was observed in three days, *i.e.*, as a result of two daily implants, in most of the animals. In the female this consisted of a swelling of the vulva and was usually followed by the opening of the vagina after three to six daily implants. In the male the testes usually showed some enlargement and tendency to become scrotal after two implants, and successive implants usually produced still greater enlargement and migration into the scrotum, which usually became more pigmented. The changes in the male were not so pronounced as in the female, for the testes rarely became as large as normal if the implants were made at other times than the breeding season. These genital changes usually persisted about two weeks or more, but they resulted in no cases of reproduction.

Daily implantation of a rat pituitary into each of 8 male and 8 female ground squirrels was begun June 7. By killing one pair on June 8 and each day thereafter a series was obtained, one pair having received one implant each, another pair, two implants, etc., up to a pair which had received eight daily implants each. The ovaries of the females showed a general increase in size, although not showing a perfectly graded series from the one to eight implants, probably because of differences in the animals before the implants were made. A control female, which had not received any implants, and also the experimental female which had received three implants had corpora lutea apparently of œstrus, since no indications of pregnancy were found. This implanted animal therefore did not fit into the series. The ovary of the animal which had received only one implant of one rat pituitary gland contained: numerous small oögonia, without surrounding follicular cells, near the periphery of the ovary; numerous small atretic follicles; and a few moderately large follicles. The animals which had received two and four daily implants showed: an increase in size and number of the larger follicles; some reduction in number of atretic follicles; and possibly some reduction in the number of peripheral oögonia. No corpora lutea were yet present, but a few of the larger follicles in the animal receiving two implants contained blood. The chief change in the animals receiving 5, 7 and 8 daily implants was the tendency of the large follicles to become corpora lutea, and this included even the ones filled with blood. The atretic follicles and also the peripheral oögonia remained about the same in number and size or were possibly reduced in number. Apparently the implants had little effect on the oögonia and the atretic follicles in eight days, but produced a striking increase in size of large or medium sized

PLATE II

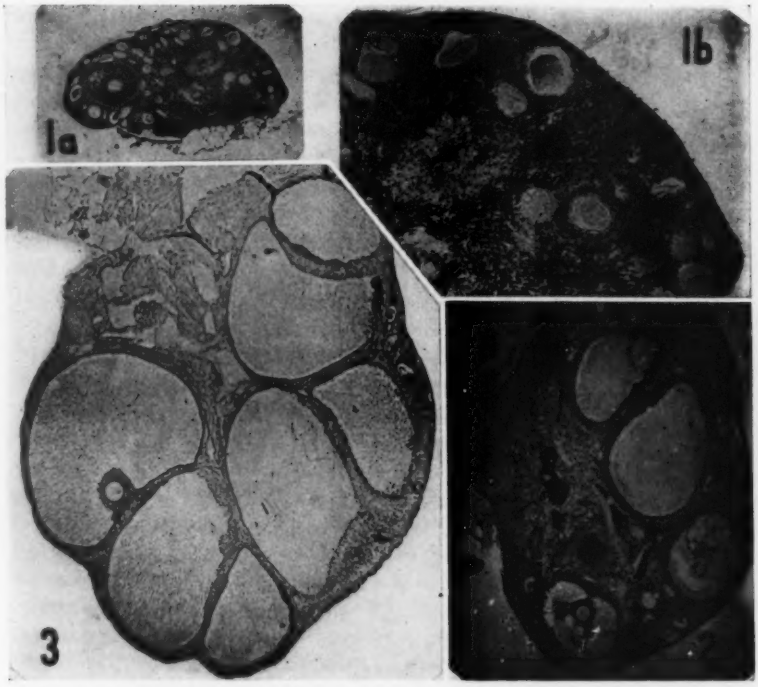


FIG. 1. *a*. A section through a portion of the right ovary of ground squirrel No. 1011 before receiving implants, × 23. *b*. A portion of the same section, × 83. The large follicles measure about 340 microns in diameter.

For further description of the figures see the text.

All the photomicrographs were taken by Mr. Charles Dobrovolny.

FIG. 2. A section through the remainder of the right ovary of ground squirrel No. 1011 after receiving 6 daily implants of rat pituitaries, × 23. The larger follicles measure about one mm. in their greatest diameter.

FIG. 3. A section through the left ovary of ground squirrel No. 1011 after 8 daily implants of rat pituitaries, × 23. The larger follicles now measure about 1.2 to 1.5 mm. in their greatest diameter.

follicles, many of which were hemorrhagic, and finally their development into corpora lutea.

More striking results were obtained from histological study of the ovaries of two females which served as their own controls. On August 8 half of the right ovary of each was removed for study of their normal condition. Each received an implant of a rat pituitary daily from August 13 to 21. The remainder of the right ovary was removed on the seventh day (after 6 pituitary implants) and the left ovary taken on August 22, the ninth day (after 8 daily implants). The normal or control piece of the right ovary of one animal contained a few oögonia without follicles, some atretic follicles and a few young to medium-sized follicles (Fig. 1). After 6 days of implantation the follicles increased in size and some of them were then mature (Fig. 2); after 8 implants these were extremely large, much larger than normal mature follicles, and some contained red blood cells in a part of the follicular cavity (Fig. 3). No marked change in number of atretic follicles and of oögonia could be noted.

The control piece of ovary removed from the other animal, August 8, contained many oögonia without follicles, very many atretic small follicles, some small follicles and some which had grown to the stage of beginning of cavity formation. Six days of implantation produced a few large follicles, most of which contained blood. After eight days the follicles were still larger, some of them containing blood, and corpora lutea were beginning to form, even beginning to hem in the blood in some of the follicles. Two ova were seen in one fallopian tube of this animal. In this and also in the other female killed August 22, one day after each had received eight daily implants of rat pituitaries, the ovaries and uteri were much enlarged and the vaginæ were swollen and open at the time of autopsy.

The males killed, one each day for 8 days, after one to eight days of pituitary implantation beginning June 8 did not show a perfectly graded series as to histological development of the testes, possibly because of variations in condition at the beginning of the experiment. A non-implanted male, killed at this time as a control, showed many more spermatogonia and more spermatogonial divisions than the implanted males, and many primary spermatocyte (spireme) stages, but without more advanced stages of mitosis. The animals implanted one and two days showed little advance over the control. Those implanted three and four days showed testicular enlargement and some descent of the testes into the scrotum, and sections of the testes showed chiefly spermatids and attached spermatozoa in addition to spermatogonia. Metamorphosing spermatids were seen in the four-day implanted ani-

mal. In these two males implanted for three and four days, the prostates and Cowper's glands were large, but this was not the case in any of the other 6 males. Probably these two males were more sexually active before the implantations than the others were, otherwise these two should not have been the only ones to show enlargement of these glands. The last four males implanted showed enlargement of the scrotum and some enlargement of the testes but not as marked as in the ones implanted three and four days. A gradual histological change occurred in these four males from a predominance of primary spermatocyte spiremes (after 5 days of implantation) through some stages of active mitosis of the first maturation division (6 and 7 days), to stages of active mitosis of the second maturation division (8 days). Spermatogonia were present, but spermatids and spermatozoa were not found in these four animals.

From each of two males, which had been in the animal house a year, a small piece of testis was removed for study of the testicular condition before pituitary implantation, August 9. These control pieces contained spermatogonia and many primary spermatocyte spireme stages without more advanced mitotic stages (Fig. 4). After four days of healing these two males were given daily implants of rat pituitaries for 8 days and the testes fixed on the ninth day, August 21. The testes of one of the males now contained many spermatids and active division phases of mitosis of maturation (Fig. 5). The testes of the other male now showed a few spermatids and several active division phases of mitosis of maturation (Fig. 6). No metamorphosing spermatids and no spermatozoa were present in either male.

While the development in the gonads was not uniformly progressive in the eight-day series of animals, it is apparent that the implants in the female generally stimulated the growth of the follicles to an unusual size, often accompanied by bleeding into the follicle; and in the male produced a general enlargement of the testes tending towards the formation of spermatids, but without enlargement of the prostate and Cowper's glands to the extent found in breeding males.

Work on the conditions of the reproductive organs at different seasons, and on the effect of pituitary implantation at different times of the year on our laboratory supply of ground squirrels, is being continued by the senior author and Mr. Mark A. Foster.

Ultra-violet Radiations

Saidman (1924) found ultra-violet light beneficial in treating menstrual disturbances. At the suggestion of Dr. R. K. Nabours of this department a mercury arc ultra-violet lamp was placed at a distance

of three to four feet above several all-wire cages containing ground squirrels. The lamp transmits slightly more ultra-violet light than is found in sunlight, and will cure rickets in chickens in half-hour daily radiations according to Professor J. S. Hughes of the Chemistry Department of this College. However, radiations of five to twenty-four hours daily had no influence on the genital or general physiological condition of our ground squirrels.

A combination of ultra-violet radiation with pituitary implantation produced no effects other than those of implantation alone.

Ovarian Extract Injections

Œstrus was produced in old albino rats by Slonaker (1927) and in castrated and normal mice by Tuisk (1927) with follicular fluid extracts. Golding and Ramirez (1928), by the use of ovarian and placental extracts, caused the vaginæ of rats to open prematurely with a production of continuous œstrus. Work by Allen and Doisy (1923) and others also show that ovarian extracts may have a marked effect upon genital changes.

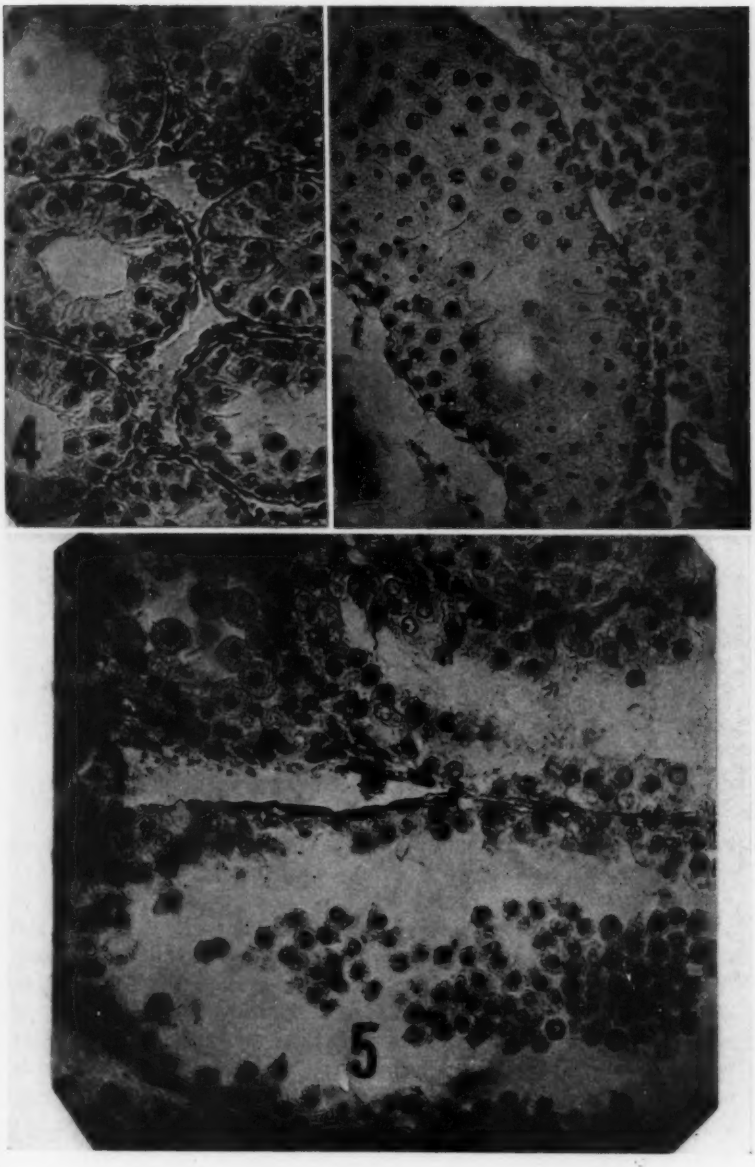
Through the courtesy of the Veterinary Division of this College we procured an alcoholic saline extract of beef ovaries from which the corpora lutea had been removed. This extract had been used with success upon non-producing cows by McLeod (1929) and Frank (1929). Injections of one cc. of this extract every third day for the last two weeks in June produced no positive changes in female ground squirrels either with the vagina open or closed, although the dosage was about 200 times as great as that used for cows when relative weights are considered. The females with open vaginæ were placed daily with sexually active males, but no reproduction took place.

Another experiment was begun January 14, 1931. The extract was injected into three female ground squirrels, one cc. daily for 22 days. It produced no apparent effect on the vagina, which was closed and not swollen at the beginning of the experiment. Histological examination of one-third to one-half the right ovary taken from each animal and from one control January 14 showed a large number of young oögonia (primitive ova) peripherially, many young follicles with one row of follicular cells, several small atretic follicles, and a medium number of mature follicles about 300 to 360 microns in their greatest diameter.

Fig. 4. A section through a portion of the right testis of ground squirrel No. 1115 before it received any pituitary implants, × 120.

Fig. 5. A section through the remainder of the right testis of ground squirrel No. 1115 after receiving 8 daily implants of rat pituitaries, × 120.

Fig. 6. A section through a testis of ground squirrel No. 1078 after 8 daily implants of rat pituitaries, × 120.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Careful histological study of the remaining half or two-thirds of the ovary after 13 daily injections showed no marked or consistent change in the two experimentals surviving and in the saline injected control. No histological change was found after 21 days of injection in the one surviving experimental and in the control. In both animals the left ovary was slightly larger than the right had been at the beginning of the experiment, and the uterus had increased about fifty per cent in diameter. Both of these conditions may probably be attributed to the approach of the spring breeding season. The health of these animals was good, the two deaths being produced by the animal chewing into the incision or the somewhat inflamed areas where the injections had been made.

Ovarian Implantation

Ovarian transplantation in the hands of other workers has had marked effect upon the recipients. Grunert (1927) produced œstrus and pregnancy in cows with homeotransplants. Sippel (1924) reported pregnancy in four women after ovarian grafts, and Tuffier and Bour (1925) reported improved health and return of menses with the possibility of pregnancy after such implants. Pettinari (1925) reactivated an old female dog with ovarian grafts.

These and other reports suggested the possibility that implants of ovarian tissue might stimulate genital functions. It was not expected that the tissue would grow, partly because rat ovaries were used, but that it would release the contained hormone as the tissue was gradually absorbed, as was the case with the pituitary tissue implants. The method and technic were practically identical in the two cases. The animals were implanted every third day for two weeks.

The four females used showed no genital development or changes. These implants were not tolerated as well as the pituitary implants, but no serious ill effects were produced.

Outdoor Cages

Since taking ground squirrels from their native habitat into the laboratory stopped their reproduction, it would seem that they should reproduce if placed in outdoor cages. Six pairs kept in such cages during the month of June showed no genital changes when dug out of their burrows. Whether reproduction would take place if the animals were in outdoor cages in April when most of the mating takes place is not known. Even if they did, this would not show why reproduction did not occur in the laboratory.

Discussion

The Possible Inhibitors of Reproduction in the Laboratory

Since the use of the mercury arc vapor lamp did not aid reproduction, the lack of sunlight would not seem to be an inhibitor of it. The benefits of the outdoor sun should last for some time after the animal is captured. Furthermore, it may be stated that the Columbian ground squirrels mate early in the spring before they have had time to absorb much ultra-violet light (Shaw, 1926). This would be true to some extent for the thirteen-lined ground squirrels, for they have a period of rut of about two weeks, shortly after coming out of hibernation, according to Drips (1919).

As the various vitamins were provided in the diet, lack of reproduction could not be attributed to their absence. The good condition of the animals, the good growth made by young ground squirrels, and also the satisfactory rate of growth and reproduction in the mice and rats fed the same diet give further evidence in that direction.

It cannot be stated that hibernation is not a necessary forerunner of reproduction, but at least it does not greatly aid reproduction in the laboratory, for great numbers of ground squirrels have been received before and during the normal breeding season (April and May), but only two known cases of copulation have occurred after they reached the laboratory, although the females were nearly always left with males. Such animals had, of course, passed through normal hibernation in nature. Ground squirrels which had hibernated in the refrigerator for varying lengths of time never reproduced following their return to the animal house.

While marked development of the reproductive organs was produced in the ground squirrel by the anterior lobe principle in the pituitary implants, this development was not as complete as that obtained by Smith and his associates in the rat and mouse, or by Wolf (1929) in the frog. Ovulation did occur in one of the implanted ground squirrels in August. Possibly the female would have reproduced at this time if the males had not been devoid of spermatozoa. Incidentally, the ovulation in this female suggests that under these conditions at least, ovulation may take place independently of copulation. Drips (1919) stated that ovulation occurs only after coitus as found for the ferret (Marshall, 1922) and rabbit (Hammond and Marshall, 1925). She also stated that corpora lutea persist throughout the summer and prevent the recurrence of œstrus after one litter is born.

Indications were found both in the literature and in our observations that the nervous state of an animal may have a profound influ-

ence on reproduction. Borries (1929) reported menstrual irregularities in 27 per cent of 39 college women because of nervous strain. Lafora (1923) considered that sexual frigidity in women was often produced by psychic inhibition. According to Steive (1926, 1927) confinement usually results in sterility and gonadal degeneration in wild animals. Testicular atrophy has been noted in men in prison and war amenorrhea in women has been produced by anxiety and worry. Sterility in women was attributed to nervous strain by Macomber (1924). Dr. Yoder of the State Hospital, Kalamazoo, Michigan, in a letter states that there is a positive correlation between genital functional derangement and insanity.

Observations made on wild rats, captured to supply pituitaries for the implant work, have indicated that nervous conditions produced by confinement tended to inhibit reproduction. These rats would fight so violently that only one or two could be kept safely in a cage. A number of these, where physical injury was eliminated, died from no apparent cause other than nervousness. A few of the tamest of these wild rats were kept for several months in cages, some with wild, and some with white rats, of the opposite sex, but without producing. The two strains will mate, however, for it was found that an escaped albino had mated with a gray male before she was captured again. A gray female, pregnant when caught, gave birth to 6 young but killed them.

The ground squirrels with which we are working never become tame in our laboratory. Gloves are practically a necessity in handling them, since they resist capture and bite freely when cornered. It is interesting to note that those handled most bite the most and those handled least are the least wild. One animal received in the spring was unusually tame but in spite of frequent handling became as wild as the others in a few weeks.

In reviewing our experimental work, we find that all of the methods used to stimulate reproductive activity, including those found valuable in other animals, were largely unsuccessful in the ground squirrels. The ineffectiveness of these methods together with the nervous condition of the animals, taken with the influence of the nervous system on genital function in other animals, suggests strongly that the almost complete failure of the ground squirrels to reproduce in the laboratory can be attributed to a nervous inhibition. If this is true, the nervous condition probably acts indirectly through the endocrine system. It is, furthermore, uncertain if larger doses of the hormones of the proper endocrine organs can override this inhibition. The cause of these abnormal genital conditions may lie in the nervous and endocrine systems working together to interfere with one or more steps in the normal reproductive process.

Summary and Conclusions

1. The ground squirrel, *Citellus tridecemlineatus*, failed to reproduce in nearly all cases under usual laboratory conditions.

2. The diet provided the animals contained all the vitamins which have been found to be necessary for reproduction.

3. Pituitary implants from rats, with or without ultra-violet radiation, did not cause reproduction but did stimulate the uterus and follicles in the ovary to excessive growth and corpus luteum formation. In the male the normal reproductive condition was not obtained at other times than the breeding season, but the implants produced a marked change in the histology of the testes from spireme stages of primary spermatocytes to spermatocyte division stages and spermatids.

4. Ovarian implants, ovarian extract injections, ultra-violet radiations, and keeping animals in outdoor cages caused no apparent effect on the genital functions.

5. The cause of the inhibition of reproduction and of the failure to reactivate the animals by various means may lie in a complex linkage of the endocrine and nervous systems.

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A NEW PENTAMEROUS HYDROMEDUSA FROM THE TORTUGAS

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During the month of July, 1929, a radially symmetrical pentamerous leptomedusa was present in very large numbers in the waters off the Dry Tortugas, Florida. This medusa was quite similar to *Pseudoclytia pentata*, described by Dr. A. G. Mayer from the same waters; in fact, it was identical with *P. pentata* in general form, in color, in habits, and in time of appearance. It differed from *P. pentata*, however, in certain important and specific structural characters, to be described below.

Mayer (1910) says of *P. pentata* that it was "exceedingly abundant at Tortugas, Florida, from June to August, 1897 to 1904. In 1905 it was relatively rare . . . and not a single specimen could be found in 1908. . . . In 1909 it again appeared in fair numbers." The animal was thus abundant, in its season, for seven successive years, then rare or not present for four successive years, reappearing in the next year. No extensive recorded towings were made at the Tortugas from 1909 to 1929. Upon the reinvestigation of the pelagic fauna in 1929 (Grave and Burkenroad, 1929), a pentamerous medusa was found to be present during July in such numbers as to be the dominant plankton form, but this medusa was found to be not specifically identical with the form described by Mayer. *Pseudoclytia pentata* was not taken at any time during the Laboratory season, from June 1 to August 18, 1929, while the medusa taken in 1929 had not been found by Mayer in the course of many years of intensive investigation. The fact that so similar a form appeared at the time when *P. pentata* might have been expected to appear suggests the rapid and complete replacement of one species by a related one, perhaps in the usual manner of invasion by a species whose range is thus extended, but perhaps—and this seems a most interesting possibility—through the complete replacement of a parent stock by a successful, recent, and local mutant from that stock. Mayer considered his *Pseudoclytia* as probably derived by mutation from some four-rayed *Clytia*-like ancestor. Both in 1899 and 1909 he found *P. pentata* to be extremely variable, although the variations do not appear to have been in the direction of the

medusa which was present in 1929. This last-found form was also extremely variable, and its aberrations appeared to be in the same direction and manner as those of *P. pentata*. The description of the medusa which was present in 1929 follows:

Pseudoclytia longleyi,[1] n. sp.

Adult Medusa.—Bell flatter than a hemisphere, 4 to 8 mm. in diameter. Gelatinous substance of the bell thin and tenuous. Fifteen to 20 simple tentacles with fairly well-developed, roundly conical basal bulbs. Tentacles, when extended, twice the bell radius or more in length; often carried contracted in close helical coils. Numerous shaftless permanently rudimentary tentacle bulbs which vary in degree of development from slight swellings of the ring-canal to bulbs as

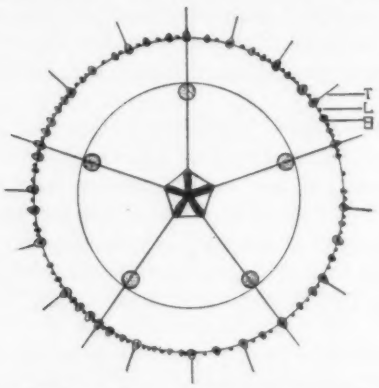

FIG. 1. Pseudoclytia longleyi, n. sp. Diagrammatic. Typical arrangement of the tentacles (*T*), rudimentary bulbs (*B*), and lithocysts (*L*). Tentacle shafts not shown full-length.

large and as well-defined as those bearing shafts; from one to five bulbs, usually two or three, between each pair of tentacles. One to three lithocysts, usually one, between each pair of tentacles and tentacle bulbs, so that the usual total number of lithocysts is about fifty. Each lithocyst contains a single spherical concretion. Velum well-developed, wide. There are five straight, narrow radial canals, 72 degrees apart. The five short, small gonads, of oval outline in the male, circular in the female, are situated upon the radial canals at points closer to the bell-margin than to the manubrium. Manubrium flask-shaped. Stomach pentagonal when viewed from the oral or aboral surface of the medusa. Five simple recurved lips, with a

[1] Named for Dr. W. H. Longley.

thin line of nematocysts along their edges. Entoderm of the stomach and gonads, translucently milky. Basal bulbs of the tentacles, and rudimentary bulbs, with a dark entodermal mass. There is an occasional variant, as in *P. pentata*, with brick-red entodermal pigment in the radial canals, tentacle bulbs, and manubrium.

The number and arrangement of the tentacles, rudimentary bulbs, and lithocysts is very variable; even different interradial sectors of the same medusa may differ greatly in this respect. There is often some unevenness in the distribution of the rudimentary bulbs, but without the extreme concentration in one sector described for *Pseudoclytia gardneri* Browne.

Young Medusa.—In medusæ 2 mm. or less in diameter, the gonads are not well-marked. In slightly larger specimens, the gonads are distinguishable as small rounded masses on the radial canals, in about the same position as those of the adult. There were no gonads apparent in the smallest medusa examined, which was .8 mm. in diameter. This medusa had eight tentacles, two tentacle bulbs, and five lithocysts, irregularly arranged, except that there was a tentacle at the end of each radial canal.

The polyp stages of the *Pseudoclytiæ* have not been described. Although some search was made, no hydroids attributable to this species were found. The presence of very early free stages, however, indicates that the fixed stages should be found in the neighborhood of the Tortugas.

The description of *Pseudoclytia longleyi* given above was prepared from living material examined at the Tortugas. The type, and cotypes, are deposited in the United States National Museum, and cotypes are contained in the collection of the Zoölogy Department of Tulane University.

Pseudoclytia longleyi differs from *P. pentata* Mayer (1900) in the following respects:

1. The presence of permanently rudimentary tentacle bulbs.
2. The larger number of lithocysts.
3. The smaller size of the medusa.
4. The situation of the gonads nearer to the bell-margin than to the manubrium.
5. The irregularity of the arrangement of the marginal appendages.

Pseudoclytia longleyi appears to be quite close to *P. gardneri* Browne. This medusa was described (Browne, 1904) from two specimens taken in the Indian Ocean. Mayer (1910) notes that they may have been aberrant specimens of a *Phialidium*. *P. longleyi* differs from *P. gardneri* in the following respects:

1. The larger number of tentacles, rudimentary bulbs, and lithocysts.
2. The symmetrical arrangement of the radial canals.
3. The situation of gonads nearer to the bell-margin than to the manubrium.
4. The greater diffuseness in the distribution of the rudimentary bulbs.

It has been suggested that the medusa described above may be a pentamerous variant, such as has been often noted for many leptomedusae, of *Phialucium carolinæ* Mayer. However, the following facts seem to the writer to strongly indicate that *Pseudoclytia longleyi* must be regarded as a distinct species, and not as a variant of *Phialucium:*

1. *Pseudoclytia longleyi* was present at Tortugas during 1929 in enormous numbers, while not a single specimen of the suggested parent stock, *Phialucium*, was taken. The center of abundance for *Phialucium carolinæ* seems to lie farther north, and it is reported by Mayer (1910) as only occasional at the Tortugas. It is usual, so far as the writer knows, for medusae which differ varietally from a parent stock in the number of radial canals to appear in company with the parent form as aberrant individuals.

2. There are strong structural differences between *Pseudoclytia longleyi* and *Phialucium carolinæ*, aside from the difference in the number of radial canals. Owing to the difficulty of obtaining specimens of *P. carolinæ*, this comparison has been made from the description and figures given by Mayer (1910).

Phialucium carolinæ	*Pseudoclytia longleyi*
Bell almost a hemisphere.	(1) Bell flatter than a hemisphere.
Gelatinous substance of the bell quite thick.	(2) Gelatinous substance of the bell thin and tenuous.
Rudimentary tentacle bulbs much smaller than the shaft-bearing bulbs.	(3) Rudimentary tentacle bulbs often almost as large as the shaft-bearing bulbs.
Lithocysts with two concretions.	(4) Lithocysts with one concretion.
Bell diameter, 14 mm.	(5) Bell diameter, 8 mm. or less.
Entoderm yellow-green.	(6) Entoderm milky or red.
Marginal appendages regularly arranged.	(7) Marginal appendages quite irregularly arranged.

The writer wishes to thank the Carnegie Institution of Washington for the privilege of working at the Tortugas Laboratory.

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THE EFFECT OF CERTAIN ENVIRONMENTAL FACTORS ON THE DEVELOPMENT AND HATCHING OF THE EGGS OF BLOOD FLUKES

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The digenetic trematodes have peculiarly elaborate and complicated life histories. Ordinarily, sexual reproduction in a vertebrate host produces eggs from which a free-swimming larval stage (miracidium) emerges and penetrates into an invertebrate host where repeated asexual multiplication produces a second and different free-swimming larval form (cercaria) which either directly or indirectly, with or without encystment, again infests the specific vertebrate host. Although the general outlines of this complex development have been known for many years and the life cycles of several species have been experimentally demonstrated, the details of the particular processes involved are as yet obscure. The factors operative in the development and hatching of the eggs, penetration of the larvæ into specific hosts, encystment, excystment, and other vital phenomena are almost entirely unknown.

Mattes (1926) studied the development of *Fasciola hepatica*, a species which infests the liver and gall bladder of cattle and sheep, and performed experiments to determine the factors operative in the emergence of the miracidia from the eggs. He found that water at a temperature of 12° C., or above, is necessary for development and that temperatures below − 7° C. will inhibit further development, whereas eggs can recover from exposure to temperatures not lower than − 3° C. He found that the optimum range was between 20° C. and 25° C., at which temperatures the miracidia required from two to three weeks to complete their development. At a range of 14° to 18° C., they required from three to six weeks. Mattes' results indicate also that a sudden drop in temperature may induce the emergence of fully developed larvæ. According to him, a pH of 7.5 to 8 was the most favorable H-ion concentration for development; lowering the pH below 6.5 caused more injury than raising it above 9; and a pH of 5 to 7.5 was the favorable range for hatching. Mattes believed that rain facilitates the emergence of miracidia, since it lowers both the temperature and pH of the water. He found that the miracidia within the eggs are positively phototrophic and that they die if the water becomes putrid or if it entirely evaporates.

Stunkard (1923) described blood flukes from the heart and arteries of various species of turtles and made certain observations on the development of the parasites. *Chrysemys marginata* and *C. picta* harbor species of *Spirorchis*. The flukes deposit their eggs in the blood vessels, by means of which they are distributed throughout the body, especially the visceral organs. The eggs rupture the capillaries and thus gain access to the tissues, through which they work their way to the alimentary tract or one of its derivatives, and ultimately they are voided with the feces. When passed by the turtles the eggs are within mucous pellets which facilitate the collection and manipulation of such minute objects. The eggs of these trematodes appear then to be particularly favorable material for study.

Material and Methods

Since information concerning the factors which influence early development and emergence of trematode larvæ are so important from a purely scientific as well as a medical and economic point of view, it seemed desirable to find out whether Mattes' results with *F. hepatica* are general and whether they are applicable to the eggs of other species. Early in October, 1929, a series of experiments was begun to determine the relation of various factors to the development and hatching of the eggs of blood flukes. The study was made on eggs voided by flukes which infect the turtles named above. Since it is not possible to make specific determination of the eggs, only generic identification is given. These turtles voided many eggs from October until the early part of the following January. For about a month, from the second week of January till the second week of February, the eggs were less numerous, but after this time they appeared again in abundance.

The procedure throughout the study was a comparatively simple one; the turtles were kept in separate aquaria and the eggs were collected daily. The greenish mucous patches, voided by the turtles and in which the eggs are embedded, were picked up in a pipette and placed in small labelled beakers containing tap water which was changed regularly to prevent putrefaction. By this method, a constant supply of eggs was obtained and those containing larvæ of different ages and from different turtles were kept separate.

When the eggs are voided, those in the same mucous pellet vary in their stage of development and in the color of the shell, which is usually a darker brown in the more advanced individuals. This variation has been explained by Stunkard (1923) by the theory that the eggs deposited in various parts of the body of the host require

different lengths of time to reach the outside. He also noted that development in the tissues of the turtle is very slow as compared with later development in water, and suggested that the more rapid later development is caused by the absorption of water and by increased oxygen supply. In the present study the development of the larvæ was followed by daily observations on the eggs. It was obviously necessary to wait until the miracidia became mature before experiments could be made to determine the effects of various factors on hatching.

Unless otherwise stated, experiments were carried on at room temperature, since previous work indicated that it is suitable for the development and hatching of these eggs.

The La Motte Standard colorimetric method was used to determine the H-ion concentration of solutions.

Observations

The earlier observations of Stunkard on the contents of the eggs and the course of development were confirmed and extended. A short time after the egg is voided and before the miracidium begins to move, vacuoles can be seen inside the shell. It is probable that these are gas vacuoles and that the gas is carbon dioxide, a product of metabolism of the larva. As development proceeds, the vacuoles become larger due to fusion of the smaller ones and also to the increasing accumulation of gas as the miracidium becomes more active. When the larva is mature there is only one vacuole, often as large as the miracidium itself. This vacuole was shown in Stunkard's figure but was not described by him. The vacuole disappears as the miracidium becomes increasingly active and the question naturally arises as to what has become of the gas and the pressure that must have been caused by it. It seemed possible that the disappearance of the vacuole might be associated with the opening of the shell, but in many instances the vacuole disappeared before the shell gave any evidence of opening. We are not at all certain as to the nature, function, and fate of this vacuole.

In the mature miracidia flame cells were regularly observed; the posterior cells are more readily perceived than the anterior ones. Flame cells have been observed beating continuously for hours, and counts show that they beat approximately 160 times per minute.

The miracidia while still inside the shell are sensitive to light, which is to be expected since they possess well-developed eye spots. The light stimulus usually requires from a few seconds to about three minutes to cause the miracidia to become active. Special precaution

was taken to ascertain that it was light and not heat from the lamp that produced the activation of the larvæ. The light was focused on a thermometer placed on the stage of the microscope in the position of the slide and after a 5-minute exposure, the mercury had risen only 0.5° C. Such a slight change in temperature, within the favorable range, could hardly cause the rapid responses manifested by the larvæ when they are stimulated by light.

The shell bears an operculum which is invisible until it starts to open. It is probable that this cap is preformed, since it is always about the same size and at one end of the shell. The removal of the operculum leaves a circular opening through which the miracidium emerges. The escape of the larva was observed many times and the process usually required about five or ten minutes, although occasionally it may take much longer. In one instance a miracidium required 1 hour and 15 minutes to emerge from the shell. The larvæ ordinarily come out anterior end first, but one was observed escaping with its posterior end first. This observation may be significant, as will be pointed out later.

When the cap opens, a fluid mass flows out of the shell along with the larva, and this mass apparently forms an envelope of some kind, for the miracidium has a difficult task to get out of it. Miracidia have been observed for as long as four hours attempting to escape from this viscous fluid. Many of them die after struggling for a long time. It has been observed that the larvæ move about just as freely inside the viscous mass outside the shell as they do inside the shell. It is possible that this substance, which gives a protein reaction, forms a rather flexible membrane when it comes in contact with water. Another possibility, although it is not supported strongly by our observations, is that this is the vitelline membrane of the egg detached from the shell and passed out of the opercular opening with the larva. It is probable that the limiting membrane rather than the viscosity of the fluid prevents the escape of the miracidia since they appear to be continually probing into it. At first the membrane merely gives under pressure and returns to its original position when the pressure is released. If the procedure is repeated often and long enough, the elasticity of the membrane is gradually reduced until it ruptures and the larva is able to escape. It has been noted that if miracidia are liberated into the water as soon as they emerge from the shell they usually disintegrate, and it is probable that during the time they are in the viscous fluid they become adjusted to life in water. If allowed to work their way out of the viscous mass or liberated after some time in it, they swim about so rapidly

that it is almost impossible to follow them. They are active for several hours under the conditions of observation.

In three experiments, eggs were placed in a small covered dish containing tap water and fecal material from the turtles. In this putrid water the embryos always died and disintegrated inside the unopened shell. On several occasions the water on the eggs was allowed to evaporate and invariably the shells collapsed and the larvæ died. It is clear that fresh water is necessary at all times.

Although a few miracidia emerged from their shells about three days after their removal from the aquaria in which the turtles were kept, the usual time required for hatching was from 5 to 7 days. Some required longer and a few remained active in their shells for as long as three weeks. Since the eggs were maintained under identical environmental conditions, the variation in time of hatching must be ascribed to other factors. Normal emergence took place at room temperature in ordinary tap water, which was changed every few days to prevent putrefaction. The pH of the water on the fifth, sixth, and seventh days was always between 7.2 and 7.6. It appears that the optimum conditions for hatching are: ordinary tap water, room temperature, and a pH zone between 7.2 and 7.6.

Effects of Acids on Development

Acetic Acid.—To test the effect of acids on development, 74 eggs of *Spirorchis*, voided during the night of November 13th, were placed in a pH 6 solution of acetic acid the following morning. On November 16th one hatched, 43 were moving in their shells, and 30 were still in early stages of development. On November 18th twelve had hatched, 32 were moving in their shells, the rest were still immature. On November 20th sixteen were hatched, 30 were moving, and 28 were in early stages of development. All of the eggs except four eventually matured and by November 26th all had hatched except the four mentioned above. It appears that development was not hastened or retarded at this pH since the time required was practically the same as when the eggs were kept in tap water. It must be recorded, however, that all of the 70 hatched miracidia died either inside the opened shell or immediately in front of the opening, outside of the shell. They did not succeed in freeing themselves from the viscous mass. The results demonstrate clearly that so long as the larvæ remain inside the unopened shell their development is not affected by the acid, but that when the shell opens, they do not long survive.

Lactic Acid.—A similar experiment with lactic acid, pH 6, gave practically identical results.

Effects of Acids upon Hatching

Eggs that had been kept in tap water until the miracidia were mature and moving actively inside their shells were used for these experiments. Since Mattes found that a slightly acid solution induced hatching of the eggs of *F. hepatica*, experiments were made to determine the effects of various concentrations of acetic, lactic, and hydrochloric acids. The first two were selected because they are frequently present at the bottom of ponds in nature.

Acetic Acid.—Mature eggs were placed in a solution, pH 6. Results are shown in Table I, and it is apparent that contact with acid

TABLE I

Effect of H-ion Concentration on Hatching of Eggs Containing Rapidly Moving Miracidia *

Acetic Acid

Time of Observance (*hours*)	pH_6 40 eggs	$pH_{6.5}$ 44 eggs	pH_7* 65 eggs	$pH_{7.5}$* 60 eggs
1/2.....	None hatched	1 hatched, but larva died almost immediately	None hatched	2 hatched
1.....	None hatched	Larvæ less active	None hatched	3 more hatched
2.....	2 shells opened, but larvæ died inside	No more had emerged	None hatched	No more hatched
3.....	1 hatched, but dead outside shell	No more had emerged	3 hatched	2 more hatched
4.....	No more hatched	No more had emerged	4 more hatched	No more hatched
6.....	2 hatched, died outside shell	No more had emerged	3 more hatched	3 more hatched
8.....	No more hatched	No more had emerged	5 more hatched	4 more hatched
12.....	Larvæ less active	No more had emerged	8 more hatched	2 more hatched
24.....	1 hatched, but dead	1 hatched, but died at once	5 more hatched	8 more hatched
30.....	No more larvæ active	15 hatched, but all were dead	None observed	Some of miracidia were swimming

* It is, of course, understood that there is little or no free acid at pH_7 and $pH_{7.5}$.

at this concentration results in the death of the larvæ. Other eggs were placed in a solution, pH 6.5. The results (Table I) show that this solution is also toxic to hatched miracidia. Tap water was added to some of the above solution to bring it to a pH of 7 and eggs containing mature larvae were placed in this fluid. The results are shown in Table I and indicate that at neutrality the solution is not toxic to the miracidia, since they hatched normally. In order to make a further test, tap water was added to some of the pH 7 solution to raise the pH to 7.5. Eggs placed in this solution also hatched normally.

When eggs were placed in the 6 and 6.5 solutions, the activity of the miracidia was greatly increased. The larvae at first swam about more rapidly and attacked the ends of the shell vigorously. After two or three hours, however, the activity of the larva had decreased to less than usual and in the pH 6 solution, especially, many of them grew weaker until they died. It appears that the acid has at first a stimulating and later a depressing and harmful effect.

Lactic Acid.—A solution of lactic acid with a pH of 6 was prepared and tap water was added to portions of it to make solutions with a pH of 6.5, 7, and 7.5. Mature eggs were placed in each of these solutions and the results are shown in Table II. The effects are not appreciably different from those obtained with similar concentrations of acetic acid. The acid is toxic and the stronger the concentration, the greater the toxicity.

An egg in which the flame cells of the miracidium were beating vigorously was placed in some of the 6.5 solution. At the end of one hour the flame cells were beating much less rapidly and at the end of three hours they were beating very feebly.

Hydrochloric Acid.—A series of flasks was prepared as before. The first contained HCl with a pH of 6, and by adding tap water to portions of this solution others were made up with H-ion concentrations of pH 6.5, 6.8, 7, and 7.5. The effects of these solutions are given in Table III. At a pH of 6, every one of the miracidia died upon coming in contact with the solution. After 24 hours the unhatched larvæ were all dead and after 72 hours they had disintegrated inside the unopened shells. In the pH 6.5 solution the unhatched miracidia were dead at the end of 48 hours. A few experiments, not recorded in the table, were done with HCl at pH 6.8. In one of them, 70 mature eggs were placed in the depression slide. At the end of 24 hours, 34 eggs had hatched. Several of the larvæ had escaped from the viscous masses, although most of them were dead inside the viscous material. In the pH 7 solution the eggs hatched normally

TABLE II

Lactic Acid

Time (*hours*)	pH_6 60 eggs	$pH_{6.5}$ 62 eggs	pH_7 89 eggs	$pH_{7.5}$ 88 eggs
1/2...	None hatched	None hatched	None hatched	None hatched
1.....	1 hatched, but died	None hatched	2 hatched	3 hatched
2.....	4 more hatched, but died	2 hatched	1 more hatched	1 hatched
3.....	No more hatched	No more hatched	2 more hatched	No more hatched
4.....	No more hatched	4 more hatched	4 more hatched	No more hatched
6.....	1 more hatched, but died	6 more hatched	8 more hatched	5 more hatched
8.....	None observed	No more hatched	6 more hatched	2 more hatched
12....	None observed	No more hatched	8 more hatched	3 more hatched
24....	22 had hatched, but all died without swimming	6 more hatched	5 more hatched	8 more hatched
30....		No observation	No observation	At 36 hours 41 eggs had hatched and most of the miracidia had become free-swimming
48....		28 eggs had hatched at this time, but all the miracidia had died inside the shell or near the opening. None had become free-swimming	At this time 66 eggs had hatched and the most of the larvæ had become free-swimming	

and the miracidia were not killed. It appears that this acid at concentrations of pH 6 and 6.5 is lethal to the larvæ and inhibits the hatching of the eggs.

TABLE III

Hydrochloric Acid

Time (*hours*)	pH6 76 eggs	pH6.5 76 eggs	pH7 69 eggs	pH7.5 58 eggs
1/2...	None hatched	None hatched	None hatched	In this experiment only 1 egg hatched in 7 days although the larva remained active inside the shells
1.....	2 hatched, but died	None hatched	1 hatched	
2.....	3 more hatched	1 hatched, but died	3 more hatched	
3.....	2 more hatched	None	2 more hatched	
4.....	3 more hatched	2 more hatched, but died	5 more hatched	
6.....	5 more hatched	4 more hatched, but died	8 more hatched	
8.....	3 more hatched	2 more hatched, but died	3 more hatched	No explanation
12....	4 more hatched	3 more hatched, but died	5 more hatched	
24...9	8 more hatched rest inactive eggs	3 more hatched, but died	6 more hatched	
48....		At this time 22 eggs had hatched, but larvæ all dead	At end of 48 hours 55 eggs had hatched	

Effects of Various Bases

A series of experiments was also carried out with the bases Na, K, and Ca (Table IV). Solutions were prepared with concentrations of pH 8 and 8.5. The results show that these solutions are all toxic, their toxicity apparently increasing in the following order; KOH, NaOH, and $Ca(OH)_2$.

Effects of Various Temperatures

A number of experiments were made to determine the limits at which these blood fluke larvæ could exist, and also the optimum temperature for development and hatching. If water in which mature eggs were placed was allowed to freeze, the miracidia died although the shells did not collapse. Fifty mature eggs were exposed to a temperature of 0° to 2° C. for 2.5 hours. When removed the larvæ

TABLE IV

Effect of H-ion Concentration and Certain Bases on Hatching of Eggs Containing Rapidly Moving Miracidia

Time (*hours*)	NaOH pH_8 66 eggs	NaOH $pH_{8.3}$ 72 eggs	KOH pH_8 62 eggs	KOH $pH_{8.8}$ 60 eggs	$Ca(OH)_2$ pH_8 85 eggs	$Ca(OH)_2$ $pH_{8.8}$ 74 eggs
1/2...	No effect	No effect	No effect	No effect	No effect	No effect
1.....	No effect	No effect	No effect	No effect	No effect	No effect
2.....	No effect	No effect	No effect	No effect	No effect	No effect
3.....	No effect	No effect	No effect	3 hatched and died, but not as soon as in strong acids	No effect	No effect
4.....	No effect	No effect	2 hatched, but dead	4 more hatched, but dead	No effect	No effect
6.....	No effect	No effect	No effect	No more	2 shells were open, but larvæ were dead in the shell	No effect
8.....	No effect	No effect	3 more hatched, but dead	8 more hatched	All quiet	No effect
12....	No effect	No effect	2 more hatched, but dead	2 more hatched	All quiet	No effect
24....	Few hatched, but dead. Rest dead in the shell	Few hatched, but dead. Rest started to disintegrate	3 more hatched, but dead. Rest dead	9 more hatched, rest dead	All dead	1 open shell, larva dead. Rest all dead in the shell and disintegrated

were quiet, but on allowing the water to reach room temperature again, they recovered and almost all of the eggs hatched during the next 48 hours. Another batch of eggs was subjected to the same

temperature for a period of five hours. None of the miracidia recovered from this exposure after being brought back gradually to room temperature. Apparently, a 5-hour exposure at this temperature range is lethal. In the next experiment 30 eggs were placed at 10° C. for eight hours. At the end of that time the miracidia were motionless, but they soon became active when returned to room temperature.

Several eggs were placed in an incubator at 25° C. for 12 hours; during which time about forty per cent of them hatched. A large number of mature eggs were placed at 36° C. for 24 hours. None of them hatched during the time, but sixty per cent of them hatched during the next 24 hours when kept at room temperature. Other eggs were kept at 40° C. for 12 hours and none of the larvæ recovered from the exposure.

Discussion

In the development of the eggs of *Spirorchis* we have described the formation of certain vacuoles which we believe contain a gas, probably carbon dioxide. In many respects these vacuoles resemble the structures described by Barlow (1925) as "oily masses" in the development of the egg of *Fasciolopsis buski*.

There are various theories to account for the opening of the operculum, none of which are entirely free from difficulties and objections. One theory postulates that the activity of the larva is sufficient of itself to force the cap off the shell. Such an explanation was advanced by Johnson (1920) for the hatching of eggs of *Echinostoma revolutum*. It is unlikely, however, that this explanation can apply to the hatching of *Spirorchis* eggs, since miracidia were observed for several days moving actively within eggs without any apparent effect upon the caps.

Another explanation is that the cap is cemented to the main body of the shell and that the cement is gradually dissolved to such a degree that the activity of the larva eventually forces the cap open. Serious objections confront this explanation also. In the course of these experiments mature eggs were kept for many days in water, in acids, and in alkaline solutions without any apparent loosening of the caps. Now if the operculum opens because the cement is dissolved by the surrounding medium, the caps should have come off all of the eggs eventually.

Another theory is that imbibition of water causes a high pressure within the shell, this pressure finally forcing the cap off. One miracidium was seen to emerge with its posterior end first, which is very unusual. By comparing this egg with others in the process of hatching and with hatched miracidia which were re-entering the shell, it was

apparent that this miracidium actually emerged with its posterior end first, and that it was not merely a case of a hatched larva re-entering the vacated shell. This observation suggests that internal pressure probably forced the cap off, and simultaneously pushed the miracidium into the opening regardless of its position. The eggs in water tend to become spherical, which indicates absorption of water. Although imbibition may cause an increase of internal pressure, the factors controlling imbibition of water are obscure.

Stunkard (1923) described two cephalic glands in the miracidium of *Spirorchis*, which open near the anterior papilla. Such glands, present in many miracidia, have been regarded by various authors as penetration glands, the secretion of which aids the larva in its entrance into a snail. It is possible that these glands, or others which open slightly behind the anterior end and secrete an oily material, becoming functional when the miracidium is mature, produce a substance which helps to dissolve the cement and loosen the cap. This theory is supported by the fact that the shells do not open until the larvæ are mature, regardless of the medium in which they are placed.

A somewhat similar observation was made by Barlow (1925) on the hatching of the eggs of *F. buski*. In that species he described the formation of a "mucoid plug" during the development of the miracidium and the application of the plug to the inside of the shell in such a manner that it covered the operculum. According to him this mass "seems to serve the purpose of protecting the operculum from the action of the secretions of the miracidium." The process of hatching was described as follows: "This is the evident action of the miracidium as it approaches the mucoid plug. It has a substance to deposit on the plug to erode it and allow of escape. It approaches with caution, applies the erosive to the plug, and then contracts vigorously several times in order to stimulate secretion. When the plug begins to get thin at the apex of the dimple, bubbles begin to show in a little line (Fig. 16) and then these coalesce to form a little tube (Fig. 17). When this tubule finally opens through, the effect on the operculum is instantaneous. No matter whether the miracidium is in contraction or in extension at the time, the operculum flies back on its hinge, water enters the egg, the miracidium becomes violently excited, ciliary motion increases to more than three hundred vibrations a minute, and the miracidium is partly extruded by the hypertonicity of the egg contents and partly assists in its own escape." The anthropomorphic interpretation of Barlow's graphic account appears to lack support, but since conditions in the eggs of *Fasciolopsis* are so different from those of *Spirorchis*, comparisons are not opportune.

The results obtained from a study of the development and hatching of the eggs of blood flukes vary considerably from those secured by Mattes for *F. hepatica*. The miracidia of the latter species require from two to six weeks to develop, whereas those of *Spirorchis* mature and emerge in about a week. The factors which induced hatching of the liver fluke eggs were not effective for those of blood flukes.

Summary and Conclusions

(1) The miracidia are positively phototrophic.

(2) The contents of the egg, which flow out of the shell with the larva, form a membrane which prevents the miracidium from escaping at once into the water. The protein in this substance may be the cause of the membrane formation.

(3) Ordinary tap water is most suitable for the development and hatching of these eggs. Putrid water is toxic to the larvæ.

(4) If the water in the culture is allowed to evaporate, the larvæ die.

(5) The most favorable pH zone for hatching of these eggs lies between 7.2 and 7.6. The miracidia cannot live in a solution having a pH lower than 6.8 or higher than 8.

(6) The optimum temperature for development and hatching is the range 20°–25° C. Exposure to temperature of 0° C. for five hours had a fatal effect upon the miracidia. Although they became quiescent at a temperature of 10° C., they recovered from it on return to room temperature. After extended exposure to temperatures higher than 40° C. the larvæ do not recover.

(7) It is postulated that conditions within the egg shell, rather than those in the surrounding medium, cause the opening of the operculum and emergence of the miracidium.

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